

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
10 May 2001 (10.05.2001)

PCT

(10) International Publication Number
WO 01/32002 A1

(51) International Patent Classification⁷: A01H 1/00, 9/00,
11/00, C07H 21/04, C12N 5/04, 5/10, 15/00, 15/09, 15/63,
15/70, 15/74, 15/82, 15/87

(21) International Application Number: PCT/US00/30503

(22) International Filing Date:
6 November 2000 (06.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/163,579 5 November 1999 (05.11.1999) US
09/693,855 23 October 2000 (23.10.2000) US

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

- With international search report.
- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/32002 A1

(54) Title: MYB TRANSCRIPTION FACTORS AND USES THEREOF

(57) Abstract: Nucleic acids that encode stress tolerance-related MYB polypeptides in plants are described. More particularly, the present invention relates to nucleotides that encode MYB transcription factors, preferably the following MYB transcription factors: MYB60, MYB74, MYB90. The present invention also relates to the MYB polypeptides themselves, as well as to variants and antibodies thereof. The invention further relates to uses of MYB transcription factors and to plants transformed by the nucleic acids. Additionally, the present invention relates to the production of stress-sensitive plants, which may be preferably used as environmental monitors.

MYB Transcription Factors and Uses Thereof

Field of the Invention

The present invention relates to nucleic acids, which encode stress tolerance-related or stress sensitivity-related myloblastosis (MYB) polypeptides in plants.

- 5 More particularly, the present invention relates to nucleic acids that encode MYB transcription factors or antisense molecules complementary to MYB transcription factors, preferably the following MYB transcription factors: MYB60, MYB74, MYB75, and MYB90. The present invention also relates to the MYB polypeptides themselves, as well as to variants and antibodies thereof. The invention further
- 10 relates to uses of MYB transcription factors and to plants transformed by the nucleic acids. The invention also relates to transgenic plants containing the MYB nucleic acids in antisense orientation.

Background of the Invention

- 15 Plant stresses such as drought, high salt concentration and high and low temperature are some of the most important factors affecting plant distribution on the earth surface. Identification of genes involved in mechanisms through which plants adapt to adverse conditions is an important goal for future improvement of crop species in their tolerance to stress, such as dehydration. Some genes involved
- 20 in water stress response present myloblastosis (MYB) recognition sites in their promoter regions. MYB proteins are a class of transcription factors, identified in nearly all eukaryotes, sharing a common DNA binding domain.

The so-called MYB domain includes two or three imperfect repeats of 50-53 amino acids (R1, R2 and R3) and is well conserved between MYB proteins of animals, yeast and plants. Although there are plant MYB-like proteins containing only one repeat, the DNA binding domain encoded by most of the plant MYB genes is formed by two repeats, which are most similar to repeats R2 and R3 of the animal cMYB proteins. Thus, MYB-related proteins from plants generally contain two related helix-turn-helix motifs, the R2 and R3 repeats. It has been suggested that MYB genes play an important role in the regulation of secondary metabolism, the control of cell shape, disease resistance, and hormone responses.

Land plants are exposed to many types of abiotic stress. One of these is dehydration, which can derive from drought, low temperature and high salt concentration in the soil. Because under those adverse environmental conditions plant growth and survival are seriously affected, series of mechanisms evolved to respond and adapt to osmotic stress. Under water-stress conditions plant cells lose water and decrease turgor pressure. The plant hormone abscisic acid (ABA) increases as a result of water stress. ABA plays an important role in the tolerance of plants to drought, high salinity and cold. Water deficit is a normal component of some developmental processes in plants, such as seed development, common to most higher plants. Such a water deficit results in changes in cell volume and membrane shape, disruption of water potential gradients and membrane integrity, protein denaturation and changes in osmolyte concentration.

The ability of plants to survive cellular water deficit depends on the species and genotype, the length and severity of water loss, the age and stage of development and the organ and cell type. Responses to water deficit may occur within seconds, such as modifications in membrane potential and in the phosphorylation status of proteins, or within minutes and hours, such as changes in protein composition and gene expression.

The first functionally characterized MYB proteins in plants, *Cl* and *Pl*, control phenylpropanoid biosynthesis in maize. Others play a role in the regulation of cell shape or in trichomes and root hair differentiation. MYB genes are involved in

the plant response to chemical messengers such as salicylic acid and hormones or in the response to different external challenges and stimuli, such as light and biotic or abiotic stresses. In general, this family participates in the control of a widespread range of functions, related to plant growth, development and interactions with the
5 environment.

It has been estimated that the plant *Arabidopsis thaliana* contains more than 100 R2R3-MYB genes. Information obtained from studying *Arabidopsis* can be applied to other flowering plants, such as those grown for fiber or food. For instance, once a gene has been discovered in *Arabidopsis*, the equivalent gene may
10 be found more easily in other plants. Thus, the function of many genes isolated from crop plants can be better understood by studying their *Arabidopsis* homologues. Thus, knowledge of *Arabidopsis* has led to a better understanding of all higher plants, and to the development of disease-resistant plants in other species.

The characterization of transcription factors that control the coordinate
15 expression of multiple genes involved in stress response is very important with respect to improving plant tolerance.

Summary of the Invention

This invention is based on the cloning of full length cDNA clones encoding
20 MYB transcription factors that result in enhanced stress tolerance in plants. The present invention also relates to the role of certain MYB genes in the control of the flavonoid and phenylpropanoid pathways. The nucleotide sequences, antisense sequences and corresponding amino acid sequences are disclosed herein.

The present invention relates to nucleic acid molecules that encode MYB
25 transcription factors, complementary antisense nucleic acids, the MYB transcription factors themselves, and variants and antibodies thereof. Preferred MYB transcription factors according to the present invention are MYB60, MYB74, MYB75, and MYB90. Certain MYB transcription factors are included in a journal article, "Towards functional characterisation of the members of the R2R3-MYB
30 gene family from *Arabidopsis thaliana*," *The Plant Journal*, 16(2), 263-276 (1998),

which is herein incorporated by reference in its entirety. The present invention also relates to uses of MYB transcription factors. Preferred uses include producing stress tolerant plants and in the case of antisense, producing stress sensitive plants that may preferably act as environmental monitors. The present invention further relates to
5 plants transformed by vectors from such nucleic acid molecules.

The present invention provides a method for genetic modification of plants to control the stress tolerance of plants, for example to drought, temperature and salt, or to increase the stress sensitivity of plants, such that they may be used as environmental monitors.

10 In one aspect, the present invention is directed to nucleic acid molecules that comprise a sequence encoding a stress tolerance-related MYB transcription factor in a plant. Preferably, the MYB transcription factor is selected from the group of MYB60, MYB74, MYB75 and MYB90. Even more preferably, the nucleic acid has a sequence that encodes one of SEQ ID NOs. 2, 4, 6 or 8.

15 In another embodiment of the invention, the present invention is directed to an isolated nucleic acid molecule that has sequence that encodes a plant stress tolerance-related MYB transcription factor. Preferably the MYB transcription factor is one of the following transcription factors: MYB60, MYB74, MYB75 and MYB90. Even more preferably, the DNA molecule hybridizes under low stringency
20 conditions with one of the following nucleic acid sequences SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7 or a variant of the isolated nucleic acid molecule.

In another embodiment of the invention, there is provided a MYB polypeptide that is a plant stress tolerance-related MYB transcription factor. A
25 preferred MYB polypeptide has the amino acid sequence of one of SEQ ID NOs: 2, 4, 6 or 8, or is a variant thereof. Also encompassed by the present invention are variants and antibodies of the polypeptides of the present invention.

The invention is further directed to a vector for transformation of plant cells.

The invention also provides a plant cell transformed with the vector as
30 described above, a plantlet, mature plant or seeds generated from such a cell, or a

plant part of such a plantlet or plant. Also provided is a method of producing a plant having enhanced stress tolerance, or in the case of antisense, producing a plant having increased sensitivity, by transforming the plant. Plants and seeds produced as described herein, or progeny, hybrids, clones or plant parts preferably exhibit

5 increased stress tolerance or increased stress sensitivity.

Further provided are methods for enhancing a plant's tolerance to stress, or in the case of antisense, increasing stress sensitivity, by transforming the plant with a vector described herein.

The nucleic acids, polypeptides, variants, antibodies, seeds and plants of the

10 present invention may also be useful as research tools. They should find broad applications in the generation of transgenic plants with enhanced tolerance to stress and enhanced sensitivity to stress.

Brief Description of the Drawings

15 Figure 1 shows a cDNA sequence encoding a AtMYB60 polypeptide (SEQ ID NO:1), as well as the corresponding amino acid sequence (SEQ ID NO:2).

Figure 2 shows a cDNA sequence encoding a AtMYB74 polypeptide (SEQ ID NO:3), as well as the corresponding amino acid sequence (SEQ ID NO:4).

Figure 3A shows a cDNA sequence encoding a AtMYB75 polypeptide (SEQ

20 ID NO:5).

Figure 3B shows an amino acid sequence (SEQ ID NO:6) of an AtMYB75 polypeptide.

Figure 4 shows a cDNA sequence encoding a AtMYB90 polypeptide (SEQ ID NO:7), as well as a corresponding amino acid sequence (SEQ ID NO:8).

25 Figure 5 shows an RT-PCR analysis of AtP5CS1.

Figure 6 shows an RT-PCR analysis of RD22.

Figure 7 shows an RT-PCR analysis of erd10.

Figure 8 shows an RT-PCR analysis of ADH1.

Figure 9 shows an RT-PCR analysis of AtMYB74.

30 Figure 10 shows an RT-PCR analysis of AtMYB75.

Figure 11 shows the expression patterns of AtMYB75, AtMYB74, ERD10, ADH1, P5CS1, and RD22, following treatment with PEG 30% and COLD 4°C.

Figure 12 shows an RT-PCR analysis of AtMYB90.

Figure 13 shows an RT-PCR analysis of AtMYB60.

5 Figure 14 shows the expression patterns of AtMYB74, ADH1, P5CS1, ERD10, RD22e, and AtMYB60, following treatment with ABA 100μM.

Figure 15 shows a comparison between the expression of MYB75 and MYB90 genes and structural genes of the phenylpropanoid pathway after light treatments. Specifically, Figure 15 shows MYB75 and MYB90 expression patterns
10 in response to white, blue, UV-A and UV-B light.

Figure 16 shows a metabolic pathway of phenylpropanoid and how MYB75 and MYB90 are believed to be involved in the pathway.

Detailed Description

15 Identification of genes involved in mechanisms through which plants adapt to adverse conditions such as drought causing conditions, may improve crop species in their tolerance to stress, such as dehydration and high salt conditions, and may thus, increase the yield of a crop. Some genes involved in water stress response present MYB recognition sites in their promoter regions. MYB proteins are a class
20 of transcription factors, identified in nearly all eukaryotes, sharing a common DNA binding domain that is highly conserved in all eukaryotes. The binding domain consists of different repeats of a helix-turn-helix motif. In animals these factors represent a small gene family involved in the control of cell proliferation and in the prevention of apoptosis. In plants these proteins form the biggest regulatory family
25 so far known, with more than 100 members identified in *Arabidopsis thaliana*, whose functions remain mainly unknown.

Applicants have identified certain MYB genes, including MYB60, MYB74, MYB75 and MYB90, which are particularly useful with regard to manipulation of stress tolerance and stress sensitivity in plants. The expression of certain genes

(*erd10*, *rd22*, *ADHI* and *AtP5CS1*) known to be involved in osmotic stress response are also described herein.

Full length cDNA sequences encoding MYB transcription factors relating to stress tolerance have been isolated by reverse transcriptase mediated polymerase chain reaction (RT-PCR). These sequences are provided herein.

Additionally, Applicants believe that the MYB75 and MYB90 genes are involved in the control of the flavonoid and anthocyanin pathways, that MYB74 is a transcription factor that is activated during stress, and the MYB60 is a transcription factor that is repressed during stress.

10

Definitions

As used herein, the term "plant" refers to either a whole plant, a plant part, a plant cell or a group of plant cells or progeny of any thereof. This term includes, but is not limited to, whole plants, plant parts, plant cells, plant organs, plant seeds, plant progeny, propagules, protoplasts, callus, cell cultures and any groups of plant cells organized into structural and/or functional units. The type of plant which can be used in the methods of the invention is not limited and includes, for example, ethylene-sensitive and ethylene-insensitive plants; fruit bearing plants such as apricots, apples, oranges, bananas, grapefruit, pears, tomatoes, strawberries, avocados, etc.; vegetables such as carrots, peas, lettuce, cabbage, turnips, potatoes, broccoli, asparagus, etc.; flowers such as carnations, roses, mums, etc.; agronomic crops such as corn, rice, soybean, alfalfa and the like; and in general, any plant that can take up and express the DNA molecules of the present invention. It may include plants of a variety of ploidy levels, including haploid, diploid, tetraploid and polyploid. The plant may be either a monocot or dicot.

The term "plant" also includes tissue of a plant in planta or in culture. Plant parts include, but are not limited to, leaves, stems, roots, and flowers. Plant cell progeny should be understood as referring to any cell or tissue derived from plant cells including callus; plants; plant seed; pollen; plant embryos; and plant parts such as stems, roots, fruits, leaves or flowers. Propagules should be understood as

referring to any plant tissue capable of being sexually or asexually propagated, or being propagated *in vivo* or *in vitro*. Such propagules preferably consist of the protoplasts, cells, calli, tissues, embryos or seeds of the regenerated plants. The use of the term "plant" in conjunction with, or in the absence of, any specific type of plant as listed above or otherwise embraced by this definition is not intended to be exclusive of any other type of plant, plant part or progeny thereof.

The term "transgenic" refers to organisms (plants or animals) into which new DNA sequences are integrated. A "transgenic plant" is defined herein as a plant which is genetically modified in some way, including but not limited to a plant which has incorporated heterologous or homologous stress tolerance-related nucleic acid molecule, such as DNA or modified DNA, into its genome. The altered genetic material may encode a protein or antisense molecule, for example. A "transgene" or "transgenic sequence" is defined as a foreign gene or partial sequence which has been incorporated into a transgenic plant.

The term "hybridization" as used herein is generally used to mean hybridization of nucleic acids at appropriate conditions of stringency as would be readily evident to those skilled in the art depending upon the nature of the probe sequence and target sequences. Conditions of hybridization and washing are well known in the art, and the adjustment of conditions depending upon the desired stringency by varying incubation time, temperature and/or ionic strength of the solution are readily accomplished. See, for example, Sambrook, J. et al., *Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor Press, Cold Spring Harbor, New York, 1989. The choice of conditions is partly dictated by the length of the sequences being hybridized, in particular, the length of the probe sequence, the relative G-C content of the nucleic acids and the amount of mismatches to be permitted. Low stringency conditions are preferred when partial hybridization between strands that have lesser degrees of complementarity is desired. When perfect or near perfect complementarity is desired, high stringency conditions are preferred. For typical high stringency conditions, the hybridization solution contains 6X S.S.C., 0.01 M EDTA, 1X Denhardt's solution and 0.5% SDS. Hybridization is

carried out at about 68°C for about 3 to 4 hours for fragments of cloned DNA and for about 12 to about 16 hours for total eukaryotic DNA. For lower stringencies the temperature of hybridization is reduced to about 42°C below the melting temperature (T_M) of the duplex. The T_M is known to be a function of the G-C content and duplex length as well as the ionic strength of the solution.

"High stringency conditions" should be understood to be those conditions normally used by one of skill in the art to establish at least about a 90% sequence identity between complementary pieces of DNA or DNA and RNA. Lesser sequence identity, such as at least about 50% sequence identity or preferably at least about 70% may also be desired and obtained by varying the hybridization conditions such that the conditions are "low stringency conditions".

As used herein, the term "substantial sequence identity" or "substantial homology" is used to indicate that a nucleotide sequence or an amino acid sequence exhibits substantial structural or functional equivalence with another nucleotide or amino acid sequence. Any structural or functional differences between sequences having substantial sequence identity or substantial homology will be *de minimis*; that is, they will not affect the ability of the sequence to function as indicated in the desired application. Differences may be due to inherent variations in codon usage among different species, for example. Structural differences are considered *de minimis* if there is a significant amount of sequence overlap or similarity between two or more different sequences or if the different sequences exhibit similar physical characteristics even if the sequences differ in length or structure. Such characteristics include, for example, ability to hybridize under defined conditions, or in the case of proteins, immunological crossreactivity, similar enzymatic activity, etc. For example, DNA or amino acid sequences having substantial sequence identity may share about 50% to about 100% sequence identity, preferably about 65% to about 99% sequence identity, and most preferably about 70% to about 99% sequence identity. Sequence identity determinations can be performed for example, using the FASTA program (Genetics Computer Group Madison, Wis.). Alternatively, identity similarity determinations can be performed using BLASTP

(Basic Local Alignment Search Tool) of the Experimental GENINFO Blast Network Service. See also Pasternak, et al. Methods in Plant Molecular Biology and Biotechnology, Glick, et al. (eds.), pages 251-267 (CRC Press, 1993). Sequence identity also includes a relationship wherein one or several subsequences of
5 nucleotides or amino acids are missing, or subsequences with additional nucleotides or amino acids are interdispersed.

The minimal amount of sequence identity required by the present invention is that sufficient to result in sufficient complementarity to provide recognition of the specific target RNA or DNA and in the case of antisense molecules inhibition or
10 reduction of its transcription, translation or function while not affecting function of other RNA or DNA molecules and the expression of other genes.

Additionally, two nucleotide sequences are "substantially complementary" if the sequences have at least about 50 percent, more preferably, at least about 70 percent and most preferably at least about 90 percent sequence similarity between
15 them. Two amino acid sequences have a substantial sequence identity if they have at least about 50%, preferably about 70% or more similarity between the active portions of the polypeptides.

The term "functional derivative" of a nucleic acid (or poly- or oligonucleotide) is used herein to mean a fragment, variant, homolog, or analog of
20 the gene or nucleotide sequence encoding a stress tolerance-related MYB transcription factor. A functional derivative may retain at least a portion of the function of the stress tolerance-related encoding DNA which permits its utility in accordance with the invention.

A "fragment" of the gene or DNA sequence refers to any subset of the
25 molecule, e.g., a shorter polynucleotide or oligonucleotide of an amino acid or nucleotide sequence that retains some desired chemical or biological property of the full-length sequence such that use of the full-length sequence is not necessary to achieve the desired purpose. A "variant" refers to a molecule substantially similar to either the entire gene or a fragment thereof, such as a nucleotide substitution variant
30 having one or more substituted nucleotides, but which maintains the ability to

hybridize with the particular gene or to encode mRNA transcript which hybridizes with the native DNA.

A "homolog" refers to a fragment or variant sequence from a different plant genus or species. An "analog" refers to a non-natural molecule substantially similar to or functioning in relation to either the entire molecule, a variant or a fragment thereof.

The term "operably linked" refers to components of a chimeric gene or an expression cassette that function as a unit to express a heterologous protein. For example, a promoter operably linked to a heterologous DNA, which encodes a protein, promotes the production of functional mRNA corresponding to the heterologous DNA.

"Functional derivatives" of the stress tolerance-related MYB polypeptides as described herein are fragments, variants, analogs, or chemical derivatives of stress tolerance-related MYB polypeptides, which retain at least a portion of the stress tolerance-related or immunological cross reactivity with an antibody specific for MYB. A fragment of the stress tolerance-related MYB polypeptide refers to any subset of the molecule. Variant peptides may be made by direct chemical synthesis, for example, using methods well known in the art. An analog of stress tolerance-related polypeptide refers to a non-natural protein substantially similar to either the entire protein or a fragment thereof. Chemical derivatives of a stress tolerance-related MYB polypeptide contain additional chemical moieties not normally a part of the peptide or peptide fragment. Modifications may be introduced into the stress tolerance-related MYB peptide or fragment thereof, for example, by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues.

A "chimeric" sequence or gene is a DNA sequence containing at least two heterologous parts, e.g., parts derived from naturally occurring DNA sequences which are not associated in their naturally occurring states, or containing at least one part that is of synthetic origin and not found in nature.

With reference to nucleic acids of the invention, the term "isolated nucleic acid" is sometimes used. This term, when applied to DNA, refers to a DNA molecule that is substantially separated from other nucleic acid sequences found in the cell. For example, the "isolated nucleic acid" may comprise a DNA molecule
5 inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a procaryote or eucaryote. Recombinant plasmids or vectors containing novel MYB genes that may be propagated in for example, *E. coli*, *S. cerevisiae* and *Agrobacteria* are contemplated for use in the present invention. These vectors may optionally contain strong constitutive promoter elements to facilitate
10 high expression of the MYB genes of the invention. Alternatively, they may contain inducible promoter elements so that expression of the MYB genes of the invention can be controlled by addition of an inducer compound.

With respect to RNA molecules of the invention, the term "isolated nucleic acid" primarily refers to an RNA molecule encoded by an isolated DNA molecule as
15 defined above. Alternatively, the term may refer to an RNA molecule that has been sufficiently separated from RNA molecules with which it would be associated in its natural state (i.e., in cells or tissues), such that it exists in a "substantially pure" form.

A "substantially pure" nucleic acid sequence is defined herein as a DNA or
20 RNA molecule (sequence) isolated in substantially pure form from a natural or non-natural source. Such a molecule may occur in a natural system, for example, in bacteria, viruses or in plant or animal cells, or may be provided, for example, by synthetic means or as a cDNA. Substantially pure DNA or RNA sequences are typically isolated in the context of a cloning vector. "Substantially pure" means that
25 DNA or RNA molecules other than the ones intended are present only in marginal amounts, for example less than 5%, less than 1%, or preferably less than 0.1%. Substantially pure DNA or RNA sequences and vectors containing may be, and typically are, provided in solution, for example in aqueous solution containing buffers or in the usual culture media.

Nucleic acid molecules of the present invention may be single stranded or double stranded or may be a DNA or RNA, or hybrids thereof.

Nucleic Acid Molecules

5 The present invention relates to a compound comprising a nucleic acid molecule that encodes a MYB transcription factor or is complementary to at least a portion of a MYB gene. The MYB transcription factor may be a stress tolerance-related MYB polypeptide. Preferred MYB transcription factors that are encoded by the nucleic acid molecule of the present invention are MYB60, MYB74, MYB75,
10 and MYB90 polypeptides. Preferably the nucleic acid is DNA that encodes an amino acid sequence having SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. A preferred embodiment of the present invention includes nucleic acid molecules that encode a MYB transcription factor, which shares about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ
15 ID NO:8. More preferably, the nucleic acid molecules encode a MYB transcription factor, which shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the nucleic acid molecules encode a MYB transcription factor, which shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ
20 ID NO:8.

Also encompassed by the present invention are isolated nucleic acid molecules having a sequence that encodes a plant stress tolerance-related MYB transcription factor. Preferably the MYB transcription factor is one of the following transcription factors: MYB60, MYB74, MYB75 and MYB90. Even more
25 preferably, the DNA molecule hybridizes under low stringency conditions with one of the following nucleotide sequences: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7 or a functional derivative or variant of the isolated nucleic acid molecule.

In a preferred embodiment of the invention, isolated nucleic acid molecules
30 encompassed by the present invention are those that encode a MYB transcription

factor, which shares about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. More preferably, the DNA molecules encode a MYB transcription factor, which shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the DNA molecules encode a MYB transcription factor, which shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

An aspect of the present invention disclosed herein provides for novel cDNA clones coding for MYB polypeptides. These cDNAs, or their genomic counterparts, or DNA molecules with substantial sequence identity to either, can be engineered for expression of the encoded MYB polypeptides and transformed into plants that have enhanced stress tolerance or, in the case of antisense, plants that are stress sensitive, as described herein.

MYB encoding nucleic acid molecules of the invention include cDNA, genomic DNA, RNA, and fragments thereof which may be single- or double-stranded. Thus, this invention provides oligonucleotides having sequences capable of hybridizing with at least one sequence of a nucleic acid molecule of the present invention, such as selected segments of the cDNA having SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7. Such oligonucleotides are useful as probes for detecting or isolating MYB genes in other plant species.

Also provided herein are compounds comprising antisense nucleic acid molecules encoding an RNA molecule which is complementary to at least a portion of an RNA transcript of the DNA molecule described herein above, wherein the encoded RNA molecule hybridizes with the RNA transcript such that expression the MYB transcription factor is altered. The antisense nucleic acid molecule can be full length or only a portion of the nucleic acid sequence.

The antisense nucleic acid molecule is substantially homologous to at least a portion of a DNA molecule encoding a MYB transcription factor. In a preferred embodiment, the DNA molecule encoding a MYB transcription factor hybridizes with SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7, or is

substantially homologous to at least a portion of an RNA sequence encoded by the DNA molecule encoding a MYB transcription factor. In one embodiment of the invention, the antisense nucleic acid molecule is substantially homologous to at least a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7, or the
5 RNA transcript encoded by SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7. In another embodiment, the antisense nucleic acid molecule is substantially homologous to at least a portion of the 5' non-coding portion of a DNA molecule encoding a MYB transcription factor, wherein the DNA molecule hybridizes with SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7.

10 Antisense oligonucleotides are preferably at least about six nucleotides in length to provide minimal specificity of hybridization and may be complementary to DNA or mRNA encoding a MYB transcription factor or a portion thereof. The antisense oligonucleotide may extend in length up to and beyond the full coding sequence for which it is antisense. The antisense oligonucleotides can be DNA or
15 RNA or chimeric mixtures or derivatives or modified versions thereof, single stranded or double stranded.

The action of the antisense oligonucleotide may result in alteration, primarily inhibition, of MYB expression in cells. For a general discussion of antisense see: Alberts, et al., Molecular Biology of the Cell, 2nd ed., Garland Publishing, Inc. New
20 York, New York, 1989 (in particular pages 195-196, incorporated herein by reference).

The antisense oligonucleotide may be complementary to any portion of the MYB gene. In one embodiment, the antisense oligonucleotide may be between 6 and 100 nucleotides in length, and may be complementary to the 5'-non-coding
25 sequence of the senescence-induced DHS sequence, for example. Antisense oligonucleotides primarily complementary to 5'-non-coding sequences are known to be effective inhibitors of expression of genes encoding transcription factors. Branch, M.A., Molec. Cell Biol., 13:4284-4290 (1993).

Preferred antisense nucleotides are substantially homologous to a portion of
30 the mRNA encoding MYB transcription factors. For example, introduction of the

full length cDNA clone encoding MYB transcription factors in an antisense orientation into a plant is expected to result in successful altered MYB gene expression. Moreover, introduction of partial sequences, targeted to specific portions of the MYB gene, can be equally effective.

5 The minimal amount of homology required by the present invention is that sufficient to result in sufficient complementarity to provide recognition of the specific target RNA or DNA and inhibition or reduction of its translation or function while not affecting function of other RNA or DNA molecules and the expression of other genes. While the antisense oligonucleotides of the invention comprise
10 sequences complementary to at least a portion of an RNA transcript of the MYB gene, absolute complementarity, although preferred is not required. The ability to hybridize may depend on the length of the antisense oligonucleotide and the degree of complementarity. Generally, the longer the hybridizing nucleic acid, the more base mismatches with the MYB target sequence it may contain and still form a
15 stable duplex. One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting temperature of the hybridized complex, for example.

Also encompassed by the present invention are nucleic acid molecules (sense and antisense) that may be modified at the sugar, base or phosphate. Those in the art
20 will recognize that one or more bases in a nucleotide sequence may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Modified bases may include for example, synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and
25 other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-
30 trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and

7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

Recombinant Vectors

5 The present invention is further directed to a recombinant vector from any of the nucleic acid molecules encoding the MYB transcription factors described above and to a recombinant vector from any of the antisense nucleic acid molecules described above.

 Vectors are recombinant DNA sequences which may be used for isolation
10 and multiplication purposes of the mentioned DNA sequence and for the transformation of suitable hosts with these sequences. A vector may be a plasmid, cosmid, bacteriophage, virus or any other replicating nucleic acid that has the capability of replicating autonomously in a host cell. Preferred vectors for isolation and multiplication are plasmids which can be propagated in a suitable host
15 microorganism, for example in *E. coli*. Many vectors have been described in the art which are suitable for use as starting materials in the present invention.

 The insertion of an appropriate sequence, which is capable of transcription, into such an intermediate vector results in a vector from a chimeric DNA sequence of the invention that can then be used to transform the desired plant. Alternatively, a
20 chimeric DNA sequence can be prepared and inserted into a suitable vector which is then used to transform the desired plant.

 Vectors of the present invention can be constructed by recombinant DNA technology methods that are standard in the art. For example, the vector may be a plasmid containing a replication system functional in *Agrobacterium*. Plasmids that
25 are capable of replicating in *Agrobacterium* are well known in the art. See, Miki, et al., Procedures for Introducing Foreign DNA Into Plants, Methods in Plant Molecular Biology and Biotechnology,, Eds. B.R. Glick and J.E. Thompson. CRC Press (1993), PP. 67-83.

 With regard to antisense nucleic acid molecules, the recombinant vectors for
30 transformation of plant cells, include (a) an antisense nucleic acid molecule

substantially homologous to (1) at least a portion of a DNA molecule encoding a MYB transcription factor, such as MYB60, MYB74, MYB75 and MYB90, or (2) at least a portion of an RNA sequence encoded by the DNA molecule encoding such a MYB transcription factor; and (b) regulatory sequences operatively linked to the antisense nucleic acid molecule such that the nucleic acid molecule is expressed in a plant cell into which it is transformed.

A polynucleotide sequence (DNA, RNA) is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that polynucleotide sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the polynucleotide sequence to be expressed and maintaining the correct reading frame to permit expression of the polynucleotide sequence under the control of the expression control sequence and production of the desired sequence.

Polypeptides

Also encompassed by the present invention are stress tolerance-related MYB transcription factors. Preferred MYB transcription factors of the present invention are MYB60, MYB74, MYB75, and MYB90. In a most preferred embodiment of this aspect of the invention, the MYB transcription factor has an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

In another preferred embodiment of the invention, the MYB transcription factor shares about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8. More preferably, the MYB transcription factor shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the MYB transcription factor shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

Antibodies

According to another aspect of the invention, antibodies immunologically specific for the polypeptides described hereinabove are provided. Such antibodies include antibodies of plant MYB polypeptides. Preferably, the antibody is an antibody of a MYB transcription factor. The antibody is more preferably an antibody of MYB60, MYB74, MYB75 or MYB90. In a most preferred embodiment of this aspect of the invention, the antibody is an antibody of a MYB transcription factor having an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

10 The present invention also provides antibodies, monoclonal or polyclonal, capable of immunospecifically binding to MYB proteins of the invention. Polyclonal antibodies directed toward plant stress tolerance-related MYB transcription factors may be prepared according to standard methods. In a preferred embodiment, monoclonal antibodies are prepared, which react immunospecifically with various epitopes of MYB transcription factors. Monoclonal antibodies may be prepared according to general methods of Kohler and Milstein, following standard protocols. Polyclonal or monoclonal antibodies that immunospecifically interact with MYB transcription factors can be utilized for identifying and purifying such proteins. For example, antibodies may be utilized for affinity separation of proteins with which they immunospecifically interact. Antibodies may also be used to immunoprecipitate proteins from a sample containing a mixture of proteins and other biological molecules.

With respect to antibodies of the invention, the term "immunologically specific" refers to antibodies that recognize and bind to one or more epitopes of a polypeptide of interest (for example, MYB60), but which do not immunospecifically recognize and bind other molecules in a sample containing a mixed population of antigenic biological molecules.

30

Variants

Also encompassed by the scope of the present invention are variants of plant MYB transcription factors. Preferably, the variants of the MYB polypeptides are variants of MYB60, MYB74, MYB75, and MYB90.

- 5 Variant nucleic acid and amino acid sequences of the present invention preferably are at least about 80% identical, most preferably at least about 99% identical, to a native sequence such as the native nucleic acid sequences of SEQ ID NOs:1, 3, 5 and 7, and the native amino acid sequences of SEQ ID Nos: 2, 4, 6 and 8. Most preferred are substantially pure DNA sequences as shown in SEQ ID
- 10 NOs:1, 3, 5 and 7, and substantially pure DNA sequences having substantial sequence identity to the sequences shown in SEQ ID NOs:1, 3, 5 and 7 (see Figures 1-4). Most preferred amino acid sequences are substantially pure amino acid sequences as shown in SEQ IDNOs:2, 4, 6 and 8 and DNA sequences having substantial sequence identity to the sequences shown in SEQ ID NOs: 1, 3, 5 and 7.
- 15 For fragments, the percent identity is calculated for that portion of a native sequence that is present in the fragment.

- Variants of MYB transcription factors may also include those that share about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. More preferably, the MYB transcription factor
- 20 shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the MYB transcription factor shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

- Variants may comprise conservatively substituted sequences, that is a given
- 25 amino acid residue may be replaced by a residue having similar physiochemical characteristics. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example,

substitutions of entire regions having similar hydrophobicity characteristics, are known by those skilled in the art.

Alterations of the native amino acid sequence may be accomplished by any of a number of known techniques. Mutations can be introduced at particular loci by
5 synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid incorporation, substitution, or deletion.

Naturally occurring MYB variants are also encompassed by the present
10 invention. Examples of such variants are polypeptides that result from alternative mRNA splicing events or from proteolytic cleavage of the MYB proteins of the present application, wherein the MYB-binding property is retained. Alternative splicing of mRNA may yield a truncated but biologically active MYB polypeptide, such as a naturally occurring soluble form of the protein, for example. Variations
15 attributable to proteolysis include, for example, differences in the amino or carboxyl termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the various MYB proteins.

Due to the known degeneracy of the genetic code wherein more than one codon can encode the same amino acid, a DNA sequence may vary from those
20 presented in SEQ ID NOs: 1, 3, 5 and 7 and still encode a MYB polypeptide having the amino acid sequence set forth in SEQ ID NOs: 2, 4, 6 and 8. Such variant DNA sequences may result from silent mutations (e.g., occurring during PCR amplification), and may be the product of deliberate mutagenesis of a native sequence.

25 Included within the scope of the present invention, in addition to the sequences exemplified specifically herein and enumerated in the sequence listing, are cDNA sequences which are equivalent to the enumerated sequences and cDNA sequences which hybridize with the enumerated sequences and encode a polypeptide having some degree of stress-tolerance activity of the given polypeptide.

30 Equivalent cDNA sequences are those which encode the same polypeptide

even though they contain at least one different nucleotide from the enumerated sequence. As is known in the art, the amino acid sequence of a polypeptide is determined by the nucleotide sequence of the DNA. Because of the redundancy of the genetic code, i.e., more than one coding nucleotide triplet (codon) can be used
5 for most of the amino acids used to make proteins, different nucleotide sequences can code for a particular amino acid.

cDNA sequences that hybridize with a given enumerated sequence and encode a polypeptide or protein having at least some degree of activity of the corresponding plant stress tolerance protein are those which exhibit substantial
10 sequence identity, as defined hereinabove, with the enumerated sequence such that it hybridizes with the latter under low stringency conditions. Proteins translated from these hybridizable cDNA sequences have different primary structures from proteins translated from the enumerated sequences. However, their respective secondary structures are the same.

15

Method for Enhancing a Plant's Tolerance to Stress

The present invention also relates to methods for enhancing a plant's tolerance to stress. The method includes transforming a plant with a vector, where the vector is as described above.
20 DNA transformation may be performed using any method of plant transformation known in the art. Plant transformation methods include direct co-cultivation of plants, tissues or cells with *Agrobacterium tumefaciens* or direct infection (Miki, et al., Meth. in Plant Mol. Biol. and Biotechnology, (1993), p. 67-88); direct gene transfer into protoplasts or protoplast uptake (Paszkowski, et al., EMBO J., 12:2717
25 (1984); electroporation (Fromm, et al., Nature, 319:719 (1986); particle bombardment (Klein et al., BioTechnology, 6:559-563 (1988); injection into meristematic tissues of seedlings and plants (De LaPena, et al., Nature, 325:274-276 (1987); injection into protoplasts of cultured cells and tissues (Reich, et al., BioTechnology, 4:1001-1004 (1986)).

Such transformation may occur for example, by incorporating a recombinant vector into a plant or deleting a recombinant vector from a plant. Alternatively, the transgenic plant may be transformed by the modification of a plant with a recombinant vector. Suitable recombinant vectors are described above and plants
5 are as defined above.

Plants include the plants defined above.

**Method of Producing a Transgenic Plant
Having Enhanced Stress Tolerance**

10 Also encompassed by the present invention are methods of producing a transgenic plant having enhanced stress tolerance. The method includes transforming a plant cell or cells with a nucleic acid molecule, which encodes a MYB transcription factor. The method then includes regenerating a transgenic plant from the transformed cell(s) such that the increased expression of the MYB
15 transcription factor confers enhanced stress tolerance to the plant. Preferably, the nucleic acid sequence encoding a MYB transcription factor is operably linked to a promoter, such that the expression of the MYB polypeptide is regulated by the promoter. Preferably the nucleic acid molecule is a recombinant DNA construct.

Also, preferably in this method, the MYB transcription factor is one of
20 MYB60, MYB74, MYB75 and MYB90. In a most preferred embodiment of this aspect of the invention, the MYB transcription factor has an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

In another embodiment, the MYB transcription factor may share about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,
25 or SEQ ID NO:8. More preferably, the MYB transcription factor shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the MYB transcription factor shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

30 Plants include the plants defined above. Stress tolerance includes tolerance to various stresses including drought, salt, cold, heat and the like. The nucleic acid

sequence may be a DNA or RNA sequence and may be single stranded or double stranded.

Using the methods of the invention, transgenic plants are generated and monitored for growth. Plants exhibiting increased resistance to environmental stress, e.g., decreased susceptibility to high temperature or low temperature (chilling), drought, infection, etc., and/ or increased resistance to pathogens, are selected as superior products. These superior plants are propagated.

10 **Method of Increasing the Expression of a
MYB Transcription Factor in a Plant**

The present invention also encompasses methods of increasing the expression of a MYB transcription factor in a plant. The method includes transforming a plant cell or cells with a nucleic acid molecule, which encodes a MYB transcription factor. The method then includes regenerating a transgenic plant from the transformed cell(s), such that the expression of the MYB transcription factor is increased relative to a non-transformed plant and whereby the increased expression of the MYB transcription factor confers enhanced stress tolerance to the plant. Preferably the nucleic acid molecule is a recombinant DNA construct.

Preferably in this method, the MYB transcription factor is one of MYB60, MYB74, MYB75 and MYB90. In a most preferred embodiment of this aspect of the invention, the MYB transcription factor has an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

In another embodiment, the MYB transcription factor may share about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. More preferably, the MYB transcription factor shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the MYB transcription factor shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

30 Plants include the plants defined above. Stress tolerance includes tolerance to various stresses including drought, salt, cold, heat and the like. The nucleic acid

sequence may be a DNA or RNA sequence and may be single stranded or double stranded.

Using the methods of the invention, transgenic plants are generated and monitored for growth. Plants exhibiting increased expression of a MYB transcription factor, as measured for example by resistance to environmental stress, e.g., decreased susceptibility to low temperature (chilling), drought, infection, etc., and/ or increased resistance to pathogens, are selected as superior products. These superior plants are propagated.

10 **Method of Increasing the Stress Tolerance of a Plant**

Further encompassed by the present invention are methods of increasing the stress tolerance of a plant. The method includes transforming a plant cell or cells with a nucleic acid sequence, which encodes a MYB transcription factor. The method then includes regenerating a transgenic plant from the transformed cell(s), wherein the expression of the MYB transcription factor is increased relative to a non-transformed plant and whereby the increased expression of the MYB transcription factor confers enhanced stress tolerance to the plant, thereby increasing the stress tolerance of a plant. Preferably the nucleic acid sequence is a recombinant DNA construct.

20 Preferably in this method, the MYB transcription factor is one of MYB60, MYB74, MYB75 and MYB90. In a most preferred embodiment of this aspect of the invention, the MYB transcription factor has an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

In another embodiment, the MYB transcription factor may share about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. More preferably, the MYB transcription factor shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the MYB transcription factor shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

25
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Plants include the plants defined above. Stress tolerance includes tolerance to various stresses including drought, salt, cold, heat and the like. The nucleic acid sequence may be a DNA or RNA sequence and may be single stranded or double stranded.

- 5 Using the methods of the invention, transgenic plants are generated and monitored for growth. Plants exhibiting increased resistance to environmental stress, e.g., decreased susceptibility to low temperature (chilling), drought, infection, etc., and/ or increased resistance to pathogens, are selected as superior products. These superior plants are propagated.

10

Method for Enhancing a Plant's Sensitivity to Stress

- The present invention also relates to methods for enhancing a plant's sensitivity to stress. The method includes transforming a plant with a vector encoding a polynucleotide sequence that is complementary to SEQ ID NO:1, SEQ
15 ID NO:3, SEQ ID NO:5 or SEQ ID NO:7, or to the mRNA encoded by SEQ ID NO:1 SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7, where the vector is as described above. Suitable methods of transformation are described above.

Plants include the plants defined above.

20

Method of Producing a Transgenic Plant with Enhanced Stress Sensitivity

- The present invention is further directed to a method of producing a transgenic plant having enhanced stress sensitivity. Such a plant preferably has a reduced level of MYB transcription factors, preferably MYB60, MYB74, MYB75
25 and MYB90 as compared to an unmodified plant. The method includes (1) transforming a plant with a vector, specifically a recombinant vector from any of the antisense nucleic acid molecules, as described above; (2) allowing the plant to grow to at least a plantlet stage; (3) assaying the transformed plant or plantlet for altered MYB activity and/or environmental stress sensitivity; and (4) selecting and growing

a plant having altered MYB activity and/or environmental stress sensitivity compared to a non-transformed plant.

The plants of this method are as described above. Preferably, the plant may be used as an environmental monitor.

5

A Transformed Transgenic Plant

The present invention further relates to a transgenic plant that is stably transformed. The transgenic plant is preferably stably transformed with a MYB gene or variant thereof, which is expressed so as to enhance stress tolerance in the
10 plant. The DNA may further comprise a screenable marker gene. Alternatively, the transgenic plant may be transformed by an antisense gene.

Also encompassed by the present invention are seeds transformed with a MYB gene or functional derivative or variant thereof. The seed may be transformed by the incorporation, deletion or modification of a seed, plant, plant part or progeny
15 thereof with a recombinant vector as described herein. Such recombinant vectors may be from any of the nucleic acid molecules or antisense nucleic acid molecules described herein.

Particular benefits may be realized by the transformation of plant cells or seeds with any of the nucleic acids comprising the genes described herein or variants
20 thereof. (That is, by incorporation, deletion or modification of these nucleic acids into a plant or seed).

Various methods for accomplishing the genetic transformation of plants (that is, stably introducing foreign DNA into plant) are known in the art. Suitable methods are believed to include virtually any method by which DNA can be
25 introduced into a cell, such as by *Agrobacterium* infection or direct delivery of DNA such as, for example, by PEG-mediated transformation, by electroporation or by acceleration of DNA coated particles, etc. Acceleration methods are generally preferred and include, for example, microprojectile bombardment and the like.

In the microprojectile bombardment method, non-biological particles may be

coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum, and the like.

Transgenic plants made in accordance with the present invention may be prepared by nucleic acid transformation using any method of plant transformation
5 known in the art.

Generally a complete plant is ultimately obtained from the transformation process. Plants are regenerated from protoplasts, callus, tissue parts or explants, etc. Plant parts obtained from the regenerated plants, such as leaves, flowers, fruit, seeds and the like are included in the definition of "plant" as used herein. Progeny,
10 variants and mutants of the regenerated plants are also included in the definition of "plant."

The transformation or genetic modification can effect a permanent change in the MYB levels in the plant and be propagated in offspring plants by selfing or other reproductive schemes. The genetically altered plant may be used to produce a new
15 variety or line of plants wherein the alteration is stably transmitted from generation to generation.

Method of Screening a Plant for Stress Tolerance

Also encompassed by the present invention is a method of screening a plant
20 for stress tolerance. The method includes screening the expression level of a stress tolerance-related MYB polypeptide in a plant. The plants of this method are as described above

After delivering nucleic acids, or variants thereof to recipient cells by any of the methods discussed above, the transformed cells may be identified for further
25 culturing and plant regeneration. In this method, the transformed cell or plant is selected or screened by conventional techniques. This step may include assaying cultures directly for a screenable trait or by exposing the bombarded cultures to a selective agent or agents.

In order to improve the ability to identify transformants, one may desire to
30 employ a selectable or screenable marker gene as, or in addition to, the expressible

gene of interest. Marker genes code for phenotypes that allow cells that express the marker gene to be distinguished from cells that do not have the marker. Such genes may encode either a selectable or screenable marker, depending on whether the marker confers a trait which one can select for by chemical means, i.e., through the use of a selective agent (e.g., an herbicide, or the like), or whether it is simply a trait that one can identify through observation or testing. Examples of suitable marker genes are known to the art and can be employed in the practice of the invention. For example, suitable markers may include markers that encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes which can be detected catalytically. Secretable proteins fall into a number of classes, including small, diffusible proteins detectable, e.g., by ELISA, small active enzymes detectable in extracellular solution (e.g., alpha.-amylase, beta.-lactamase, phosphinothricin transferase), or proteins which are inserted or trapped in the cell wall (such as proteins which include a leader sequence such as that found in the expression unit of extensin or tobacco PRS), of course, in light of this disclosure, numerous other possible selectable and/or screenable marker genes will be apparent to those of skill in the art. Therefore, the foregoing discussion is intended to be exemplary rather than exhaustive.

The transformed cell or plant contains the chimeric DNA sequence and is then regenerated, if desired, by known procedures, for both monocot and dicot plants. The regenerated plants are screened for transformation by standard methods. Progeny of the regenerated plants is continuously screened and selected for the continued presence of the integrated DNA sequence in order to develop improved plant and seed lines. The DNA sequence can be moved into other genetic lines by a variety of techniques, including classical breeding, protoplast fusion, nuclear transfer and chromosome transfer.

Where both an expressible gene that is not necessarily a marker gene is employed in combination with a marker gene, one may employ the separate genes on either the same or different DNA segments for transformation. In the latter case,

the different vectors are delivered concurrently to recipient cells to maximize co-transformation.

In order for a newly inserted gene or DNA sequence to be expressed, resulting in production of the protein which it encodes, or in the case of antisense DNA, to be transcribed, resulting in an antisense RNA molecule, the proper regulatory elements should be present in proper location and orientation with respect to the gene or DNA sequence. The regulatory regions may include a promoter, a 5'-non-translated leader sequence and a 3'-polyadenylation sequence as well as enhancers and other regulatory sequences.

Promoter regulatory elements that are useful in combination with the MYB gene to generate sense or antisense transcripts of the gene include any effective promoter in general, and more particularly, a constitutive promoter such as the fig wart mosaic virus 35S promoter, the cauliflower mosaic virus promoter, CaMV35S promoter, or the MAS promoter, or a tissue-specific or senescence-induced promoter, such as the carnation petal GST1 promoter or the *Arabidopsis* SAG12 promoter (See, for example, J.C. Palaqui et al., Plant Physiol., 112:1447-1456 (1996); Morton et al., Molecular Breeding, 1:123-132 (1995); Fobert et al., Plant Journal, 6:567-577 (1994); and Gan et al., Plant Physiol., 113:313 (1997), incorporated herein by reference). Preferably, the promoter used in the present invention is a constitutive promoter.

Expression levels from a promoter which is useful for the present invention can be tested using conventional expression systems, for example by measuring levels of a reporter gene product, e.g., protein or mRNA in extracts of the leaves, flowers, fruit or other tissues of a transgenic plant into which the promoter/reporter have been introduced.

Method for Increasing the Stress Resistance of a Crop in a Field

Another embodiment of the invention is a method for increasing the stress resistance of a crop in a field. The method includes planting in the field seeds or plants, such as the transgenic plants or seeds described herein, which are

transformed with the vectors described herein, by any of the methods described herein. Suitable methods of planting are known to those in the art.

Method of Inhibiting the Expression of MYB Genes in a Plant

5 The present invention further relates to a method of inhibiting the expression of MYB genes in a plant cell, the method includes integrating into the genome of a plant a vector specifically, a recombinant vector from any of the antisense nucleic acid molecules, as described above, and growing the plant. In this method, the antisense nucleic acid molecule is transcribed, such that expression of the MYB
10 gene is inhibited.

Method of Assaying the Environmental Conditions of a Field

The invention further relates to a method of assaying the environmental conditions of a field. Such a method includes planting any of the plants described
15 herein, including those transformed by the vectors described herein, both antisense and non-antisense, and monitoring the growth of the plant.

Method of Increasing the Production of Products of the Phenylpropanoid Biosynthesis Pathway in a Plant

20 Also encompassed by the present invention is a method of increasing the production of products of the phenylpropanoid biosynthesis pathway in a plant. The method includes transforming a plant cell with a recombinant DNA construct comprising a nucleic acid sequence encoding a MYB transcription factor. The method then includes regenerating a transgenic plant from the transformed cell,
25 wherein the expression of the MYB transcription factor increases the expression of genes encoding gene products affecting the phenylpropanoid pathway, thereby increasing the production of products of the phenylpropanoid biosynthesis pathway.

Products of the phenylpropanoid pathway include, but are not limited to stilbenes, flavonoids, lignins, salicylic acid, anthocyanins, phenolic derivatives and
30 the like.

Preferably in this method, the MYB transcription factor is one of MYB60, MYB74, MYB75 and MYB90. In a most preferred embodiment of this aspect of the invention, the MYB transcription factor has an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

5 In another embodiment, the MYB transcription factor may share about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. More preferably, the MYB transcription factor shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the MYB transcription factor shares about 70%
10 to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

Plants include the plants defined above. Stress tolerance includes tolerance to various stresses including drought, salt, cold, heat and the like. The nucleic acid sequence may be a DNA or RNA sequence and may be single stranded or double
15 stranded.

Using the methods of the invention, transgenic plants are generated and monitored for growth. Plants exhibiting increased expression of products of the phenylpropanoid biosynthesis pathway, as exhibited for example by decreased susceptibility to high temperature or low temperature (chilling), drought, infection,
20 etc., and/ or increased resistance to pathogens, are selected as superior products. These superior plants are propagated.

Further encompassed by the present invention is a method of decreasing the production of products of the phenylpropanoid biosynthesis pathway in a plant. The method includes (i) transforming a plant cell with a vector comprising an antisense
25 nucleic acid molecule substantially complementary to at least a portion of a DNA molecule encoding a MYB transcription factor or at least a portion of an RNA sequence encoded by the DNA molecule encoding said MYB transcription factor; and regulatory sequences operatively linked to the antisense nucleic acid molecule; such that the nucleic acid molecule is expressed in a plant cell into which it is
30 transformed; and (ii) growing the plant, whereby the antisense nucleic acid molecule

is transcribed, such that expression of the MYB gene is inhibited, thereby decreasing the production of products of the phenylpropanoid biosynthesis pathway. For example, products of the phenylpropanoid pathway such as stilbenes, flavonoids, lignins, salicylic acid, anthocyanins, phenolic derivatives and the like are decreased
5 by this method.

In the case of antisense, transgenic plants are generated and monitored for growth. Plants exhibiting an increased stress sensitivity are selected and propagated.

10 **Isolation of MYB Nucleic Acids and Construction of MYB Encoding Vectors**

Nucleic acid molecules encoding the MYB transcription factors of the present invention may be prepared by two general methods: (1) they may be synthesized from appropriate nucleotide triphosphates, or (2) they may be isolated from biological sources. Both of the above methods are well known in the art.

15 Nucleic acid sequences encoding the MYB transcription factors of the present invention may be isolated from appropriate biological sources using methods known in the art. In accordance with the present invention, nucleic acids having the appropriate level of sequence identity with the protein coding region of SEQ ID NOs: 1, 2, 4, or 5 may be identified by using hybridization and washing conditions of
20 appropriate stringency. For example, hybridizations may be performed, according to the method of Sambrook et al., (22) using a hybridization solution including: 5 times SSC, 5 times Denhardt's reagent, 1.0% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA, 0.05% sodium pyrophosphate and up to 50% formamide. Hybridization is carried out at 37-42°C for at least six hours. Following
25 hybridization, filters are washed as follows: (1) 5 minutes at room temperature in 2 times SSC and 1% SDS; (2) 15 minutes at room temperature in 2 times SSC and 0.1% SDS; (3) 30 minutes-1 hour at 37° C. in 1 times SSC and 1% SDS; (4) 2 hours at 42-65°C in 1 times SSC and 1% SDS, changing the solution every 30 minutes.

Nucleic acids of the present invention may be maintained as DNA in any
30 convenient cloning vector. In a preferred embodiment, clones are maintained in

plasmid cloning/expression vector, such as pBluescript (Stratagene, La Jolla, Calif.), which is propagated in a suitable *E. coli* host cell.

A full-length MYB polypeptide of the present invention may be prepared in a variety of ways, according to known methods. The protein may be purified from
5 appropriate sources, e.g., plant or animal cultured cells or tissues, by immunoaffinity purification.

Alternatively, according to a preferred embodiment, larger quantities of MYB polypeptide may be produced by expression in a suitable procaryotic or eucaryotic system. For example, part or all of a DNA molecule, such as the cDNA
10 having SEQ ID NO: 1, may be inserted into a plasmid vector adapted for expression in a bacterial cell, such as *E. coli*, or into a baculovirus vector for expression in an insect cell. Such vectors comprise the regulatory elements necessary for expression of the DNA in the host cell (e.g. *E. coli*, plant cell or insect cell), positioned in such a manner as to permit expression of the DNA in the host cell. Such regulatory
15 elements required for expression include promoter sequences, transcription initiation sequences and, optionally, enhancer sequences.

The MYB polypeptide produced by gene expression in a recombinant procaryotic or eucaryotic system may be purified according to methods known in the art. In a preferred embodiment, a commercially available expression/secretion.
20 system can be used, whereby the recombinant protein is expressed and thereafter secreted from the host cell, to be easily purified from the surrounding medium. If expression/secretion vectors are not used, an alternative approach involves purifying the recombinant protein by affinity separation, such as by immunological interaction with antibodies that bind specifically to the recombinant protein or nickel columns
25 for isolation of recombinant proteins tagged with 6-8 histidine residues at their N-terminus or C-terminus. Such methods are commonly used by skilled practitioners.

The MYB proteins of the invention may be analyzed according to standard procedures. For example, such proteins may be subjected to amino acid sequence analysis, according to known methods.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting to the present invention.

5 EXAMPLE 1

Genes induced during water stress conditions are not only thought to protect cells from osmotic stress but also to be involved in the regulation of genes for signal transduction in water deficit response. A first group of genes codes for proteins that directly function in stress tolerance. A second group of genes induced under water stress conditions encodes for regulatory proteins that function in signal transduction pathways. Examples are protein kinases, PLC, 14-3-3 proteins and transcription factors directly involved in the further control of gene expression during water stress response. Certain genes respond to drought, salt and cold stress at the transcriptional level. Expression patterns of dehydration-inducible genes are complex: some genes respond to water stress very rapidly, whereas others are induced slowly after the accumulation of ABA. Most of the genes that respond to drought, salt and cold stress can also be induced by exogenous applications of ABA. It is believed that dehydration may trigger the production of ABA, which in turn induces various genes. On the other hand, several genes that are induced by water stress are not responsive to exogenous ABA treatment. Analysis of the expression of water-stress-inducible genes by ABA in *ABA-deficient (aba)* or *ABA-insensitive (abi)* *Arabidopsis* mutants have indicated that some of the stress-inducible genes do not require an accumulation of endogenous ABA under drought or cold conditions. These observations suggest the existence of both ABA-independent and ABA-dependent signal transduction cascades between the initial signal of drought or cold and the further expression of specific genes. In addition, analysis of the expression of ABA-inducible genes revealed that several of them require protein biosynthesis for their ABA induction, while others do not, suggesting the existence of at least two independent pathways between the upstream production of endogenous ABA and gene expression during stress. The ABA-inducible genes that do not require protein

biosynthesis for their expression contain a potential ABA-responsive-element, termed ABRE (PyACGTTCCG) (SEQ ID NO:9) in their promoter regions. The ABRE resembles the G-box element, an ACGT "core" containing element, that functions in the regulation of plant genes in a variety of environmental conditions, such as light, UV, wounding and anaerobiosis. Basic region leucine zipper (bZIP) proteins have been shown to be involved in the binding to this class of elements. Furthermore, a coupling element is required to specify the function of the ABRE, constituting an ABA-responsive complex.

Along the second ABA-dependent pathway, protein biosynthesis is necessary for the expression of water-stress-inducible genes. A 67 bp region in the promoter of *rd22*, an *Arabidopsis* gene whose expression is mediated by ABA and requires protein biosynthesis, is essential and sufficient for its dehydration and ABA-inductibility. This region contains two closely located putative recognition sites for the basic helix-loop-helix protein MYC (CANNTG) (SEQ ID NO:10) and one for a MYB protein (PyAACPyPu) (SEQ ID NO:11). However, this region does not contain ABRE sequences.

A possible role of the *Arabidopsis* AtMYB2 in water stress response is the induction of *rd22* gene and under low oxygen conditions the induction of the *ADH1*. *Rd22BP1* gene, which encodes a MYC transcription factor, and *AtMYB2* are both induced by dehydration stress. The corresponding proteins bind *in vitro* to have the 67 bp region of the *rd22* gene promoter. These results suggest that MYB and MYC homologues are involved in the regulation of gene expression along one of the ABA-dependent signal cascade. However, the existence of several genes induced by drought and cold in *aha* and *abi* *Arabidopsis* mutants suggests the presence of signal transduction pathways that do not require ABA accumulation for their induction. A 9 bp dehydration responsive element, termed DRE (TACCGACAT) (SEQ ID NO:12) is essential for the ABA-independent induction of many stress-inducible genes such as *rd29A*, *kin1*, *cor6.6* and *rd17*, under drought, high salt and high and low temperature conditions. Concerning the ABA-independent pathways, the existence of several drought-induced genes that do not respond to cold or ABA

treatment suggest that there is at least a fourth pathway, which can be involved in the plant tolerance to environmental stress, such as dehydration. As suggested by the classes of mutants recovered that respond in different ways to ABA, cold and osmotic stress ABA-dependent and ABA-independent pathways may interact and
5 coverage to activate stress genes.

Several MYB recognition sites have been found in the promoter regions of different genes induced under osmotic stresses. Therefore, Applicants studied the possible involvement of MYB proteins in the regulation of stress induced genes. The expression pattern of four MYB genes were analyzed in response to different
10 osmotic stress. Certain putative target genes known to be induced by water stress, i.e., *AtP5CS1*, *rd22*, *erd10* and *ADH1*, were also included in this analysis to hypothetically localize these MYB transcription factors along the four different signal transduction pathways recently proposed.

Seeds of wild type *Arabidopsis thaliana* (Columbia ecotype) were used in
15 this study. For cold treatment seeds were sown on Einhietserde soil, treated at 4°C for 4 days to promote even germination, then grown with a 16-hours light/8-hours dark cycle at 22°C for 4 weeks and subsequently incubated at 4°C for up to 48 hours in the dark. The entire aerial part of the plants was collected after 2, 4, 6, 8, 24, and 48 hours.

For drought, ABA, PEG and NaCl treatments seeds were surface-sterilized
20 with ethanol for 2 minutes, then with a solution of sodium hypochlorite (0.5% v/v) for 5 minutes, rinsed 3 times with sterilized distilled water, treated at 4°C for 4 days to promote even germination. For drought treatment sterilized seeds were sown on MS medium agar (0.8% w/v) plates, supplemented with sucrose (1% w/v) and MES (0.5
25 g L⁻¹), grown with a 16-hour light/8-hour dark cycle at 22°C for 2 weeks, then dehydrated on 3MM paper at 22°C in the light for 1, 2, 3, 5, and 7 hours.

For ABA, PEG (Polyethyleneglycol 6000) and NaCl treatments plants were grown in liquid MS medium, supplemented with sucrose (3% w/v) and MES (0.5 g L⁻¹), with a 16-hour light/8-hour dark cycle at 22°C for 3 weeks in an orbital shaker,
30 then ABA (\pm cis-trans isomers) or PEG 6000 or NaCl were added at a final

concentration of 100 μ M, 30% w/v and 200 mM respectively; the samples were collected after 1, 2, 4, 6, 8, 16, 24, and 48 hours. For PEG 30% treatment after 6 hours of stress samples were re-hydrated transferring the plants in fresh medium without PEG and collected after 1, 4 and 24 hours (R1h, R4h and R24h). An untreated culture (PEG and NaCl control) and a culture treated with the solvent ethanol used for the ABA treatment (ABA control) were also harvested. In each case the plants were subjected to the stress treatments for various time periods, frozen in liquid nitrogen and stored at -80°C.

The results are shown in Table 1.

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RNA extraction and RT-PCR analysis

Total RNA was isolated from whole plants collected at various time periods of treatments by methods known to those in the art.

Reverse transcriptase polymerase chain reaction (RT -PCR) was used to detect *AtMYB75* and *AtP5CS1* genes transcripts. All RNA samples were treated with DNaseI (15 units) before cDNA synthesis. First strand cDNA synthesis was carried out from 6 μ g of total RNA with an oligo (dT) and RT Superscript™ II (300 units) as recommended by the manufacturer. The primer used was a 35-base oligonucleotide with 17dT residues and an adapter (5'-GGGAATTCGTCGACAAGC-3') (SEQ ID NO:13) sequence. First-strand cDNA was used as template for PCR amplification. Amplification reactions containing an aliquot of cDNA, 1X PCR Buffer II, 2.5 mM MgCl₂, 200 μ M of each dATP, dCTP, dGTP and dTTP, 0.1 μ M of each primer and 2.5 unit of AmpliTaq were performed in a final volume of 50 μ l. After the first denaturation step (2 min and 30 sec at 94°C), the reaction mix underwent 20 cycles of denaturation at 94°C at 45 sec, annealing at 55°C for 1 min and extension at 72°C for 2 min. A final extension at 72°C for 7 min was performed to complete the reaction. A set of primers specific for the *TSB1* gene of *Arabidopsis*, which encodes the β subunit of tryptophan synthase, were used to standardize the concentration of the different samples. The length of the amplified product was 476 bp. To ensure the amplification reactions

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were within linear ranges, the reactions were carried out for 20 cycles. The PCR products were fractionated on 2% w/v agarose gels and transferred onto Hybond N+ nylon membranes and hybridized employing probe labeled with fluorescein, according to the manufacturers' protocols. This standardization was confirmed using a set of primers specific for *Arabidopsis ACT1* gene that encodes for actin. For mRNA detection of the genes under analysis, the specific primer sets were used. The PCR products were then separated on agarose gels 2% w/v, and transferred onto Hybond N+ nylon membranes and hybridized with random primed fluorescein fragments.

Four MYB genes were studied in response to osmotic stress. Quantitative RT-PCR analysis was performed on RNA samples obtained from plants exposed to abiotic stresses like low temperature, drought, high salt, PEG and abscisic acid supply. Applicants analyzed the kinetics of expression of four MYB genes and of four putative target genes known to be induced by water stress, *AtP5CS1*, *rd22*, *erd10* and *ADH1*. The cDNA and amino acid sequences of *AtMYB60* are reported in Figure 1, of *AtMYB74* in Figure 2, of *AtMYB75* in Figure 3A and Figure 3B, and of *AtMYB90* in Figure 4.

Table 1 summarizes the results obtained for all genes analyzed.

Table 1

Schematic representation of MYB genes and *AtP5CS1*, *erd10*, *rd22* and *ADH1* genes expression under different stress conditions: drought, PEG (30%), ABA (100 μ M), NaCl (200 mM) and cold (4°C).

		Drought	PEG	ABA	NaCl	Cold
25	<i>AtMYB60</i>	---	-	---	-	0
	<i>AtMYB74</i>	+++	+++	+++	0	+
	<i>AtMYB75</i>	+++	+++	+++	+++	n.d.
	<i>AtMYB90</i>	+	0	+++	+++	n.s.
30	<i>AtP5CS1</i>	+	+++	+	0	+++
	<i>erd10</i>	+++	+++	+	+	+++

rd22	+	+	+	+	+
ADH1	+	+	+	0	+

5 Symbols indicated: **n.s.** no signal; **n.d.** not determined; **0** no induction; + slight induction; + + + high induction; - slight repression; - - - high repression

The expression of *AtP5CS1* (see Figure 5) was strongly induced within 1 hour after the initiation of drought treatment and high levels of transcript were maintained during 7 hours of dehydration. These data are consistent with
 10 previously published results where the expression of *AtP5CS1* was induced by exogenous ABA treatment. ABA supply induced *AtP5CS1* gene expression within 2 hours, reaching a peak after 4 hours, then the level of this transcript decreased gradually. In PEG treated plants *AtP5CS1* mRNA was induced in a two-phase time course: the first peak of induction was observed after 1 hour of PEG supply, then
 15 the level of expression decreased; after 6 hours mRNA accumulated once again. When plants treated with PEG for 6 hours were re-hydrated for 1, 4 and 24 hours the level of the transcript decreased gradually, returning to the level present in untreated plants. A similar two phases induction process was also observed for *rd22* (see Figure 6), *erd10* (see Figure 7) and *ADH1* (see Figure 8). The same kinetics had
 20 been previously reported for *erd10* gene upon cold stress and for *rd29A* during dehydration treatment. It has been shown that in dehydration conditions endogenous ABA began to accumulate 2 h after the beginning of the treatment and reached its maximum at 10 hours. Taken together, these results suggest that the first rapid induction in the two-step kinetics is not mediated by ABA, while the late induction
 25 is ABA-dependent. Our results from PEG and ABA treatments confirm a role for ABA in the late induction not only for *erd10* but also for *AtP5CS1*, *rd22*, and *ADH1* while early transcript accumulation seems to be ABA-independent.

Among the MYB genes analyzed only *AtMYB74* (see Figure 9) and *AtMYB75* (see Figure 10) are rapidly induced in response to PEG 30% and their
 30 transcripts are maintained at high level throughout the length of the treatments even if only *AtMYB74* shows a clear two phases induction process. Their transcripts are

also induced very rapidly by exogenous ABA and drought treatments, while their expression is differentially modulated by NaCl treatment (Table 1). Therefore, *AtMYB74* and *AtMYB75* are believed to be good candidates to regulate genes involved in water stress response along two different signal transduction pathways an ABA-independent pathway, early activated under osmotic stress and responsible for the rapid induction of *AtMYB74* and *AtMYB75* as well as an ABA-dependent pathway, activated after ABA accumulation and responsible for the second phase of induction of those genes. See the comparison of the expression patterns of *AtMYB75* and *AtMYB74* to ER10, ADH1, P5CS1 and RD22 in Figure 11.

Another gene, *AtMYB90*, phylogenetically correlated to *AtMYB75*, showed a similar pattern of expression in response to ABA and NaCl, while it is not induced by PEG treatment (see results of the RT-PCR analysis in Figure 12). In response to drought treatment *AtMYB90* transcript appears three hours after the beginning of the stress and its level is reduced with respect to that of *AtMYB74* and *AtMYB75* (Table 1). Therefore, its role in stress response is not very clear. *AtMYB60* was the only *MYB* gene analyzed that was repressed by water and osmotic stress conditions (Table 1); its transcript levels decreased significantly within 1 hour after the initiation of drought and ABA treatment and only slightly after PEG and NaCl supply (between 4 and 6 hours after the initiation of the treatments).

It is believed from these experiments that *AtMYB74* is activated by stress both by an ABA dependent and an ABA independent pathway, while *AtMYB60* is repressed by stress (particularly drought stress) in an ABA dependent manner.

EXAMPLE 2

Phenylpropanoid molecules comprise a set of important secondary products such as anthocyanin pigments, flavonoids, phytoalexins, phenolics acids which are involved in the protection of plants against UV damage, oxidative stress, pathogen attack, etc. The biochemical pathways leading to the synthesis of most of these

compounds are understood and several of the structural and regulatory genes involved have been cloned from maize, petunia and snapdragon.

In maize the enzymes involved in this biosynthesis are regulated in a coordinated way as a result of the activation of regulatory genes that are expressed in a tissue specific manner. Genetic and molecular analyses indicate that the regulatory genes can be grouped in two families, the *R/B* gene family, which encodes related proteins with a basic-helix-loop-helix (bHLH) DNA binding domain and the *C1/Pl* family, which encodes related proteins with MYB-domain. A member of each of the two families is preferably expressed for the transcriptional activation of the biosynthetic genes.

The RNA gel blot and RT-PCR analysis has revealed that light dependent anthocyanin accumulation is due to the light induced expression of the MYB *C1* and *Pl* genes.

In *Arabidopsis* the structural genes of the flavonoid pathway had previously been studied and cloned but the transcription factors regulating this biosynthesis were not previously understood. To understand the role of MYB genes in *Arabidopsis*, Applicants undertook a quantitative RT-PCR analysis performed on RNA samples of *Arabidopsis* obtained from several tissues and at different times after treatment with radiations such as white and blue light, UVA, UVB. The expression patterns were then compared with those of chalcone synthase (CHS) gene and dihydroflavonol-4-reductase (D4R) gene, structural genes of the flavonoid pathway. The expression patterns are shown in Fig. 15. The MYB-75 and MYB-90 expression patterns in response to white, blue, UV-A and UV-B light are consistent with their putative role in the control of phenylpropanoid pathway.

The expression pattern of AtMYB75 and chalcone synthase (CHS) gene, induced by white, blue, UVA and UVB light suggests that MYB 75 could regulate the expression of CHS while that of AtMYB90 and D4R induced with a similar kinetic by white, blue and UVA light suggests that MYB90 could regulate the expression of D4R. The believed roles of MYB75 and MYB90 in the phenylpropanoid metabolic pathway are depicted in Figure 16.

Among the more than 100 MYB genes cloned in *Arabidopsis*, the ones showing the highest similarity to the maize *C1* and *Pl* gene are AtMYB75 and AtMYB90. Thus, Applicants believe that AtMYB75 and AtMYB90 are transcription factors that regulate flavonoid biosynthesis.

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EXAMPLE 3

Construction and Analysis of MYB Transgenic plants.

Individually, each of the MYB genes of the present invention are placed in a sense or antisense orientation under the control of the constitutive CaMV 35 S promoter and are introduced into the tobacco cultivar Xanthi. Independent transgenic tobacco plants are generated. Vector-only transformed plants are also generated to be used as controls. The MYB transgenic plants exhibit enhanced resistance to environmental stresses as compared to the control plants.

Transgenic plants having MYB genes introduced in the antisense orientation exhibit increased sensitivity to environmental stresses, such as drought and high salt conditions, as compared to the control plants. These plants show a decrease in expression of the MYB genes that enhance resistance to the high stress conditions.

Progeny of plants having the MYB introduced in the sense and antisense orientation are each collected and further analyzed. Resistant and sensitive progeny are generated for further use.

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EXAMPLE 4

To induce anthocyanin pigmentation in maize, the contemporary expression of one member of the R/Sn gene family (bHLH transcription factors) and a member of the C1/Pl gene family (MYB) is needed. So we used a maize line carrying deletion for the *r* genes and carrying a small *pl* gene (recessive allele). In this condition no anthocyanin pigment are produced.

Applicants performed a shot gun experiment. The constructs used were cDNA of the different genes under 35S promoter. Individually, MYB75 and MYB90 genes from *Arabidopsis*, were introduced into maize mutants lacking

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anthocyanin. Applicants shot gun germinated maize seeds with the different combinations of construct and after four days we scored the seedlings for red spots. The presence of red spots is due to an accumulation of anthocyanin, which indicates that the transcription factors were expressed and able to induce the transcription of the structural genes of the anthocyanin biosynthetic pathway. The results of this experiment are set forth in Table 2 below

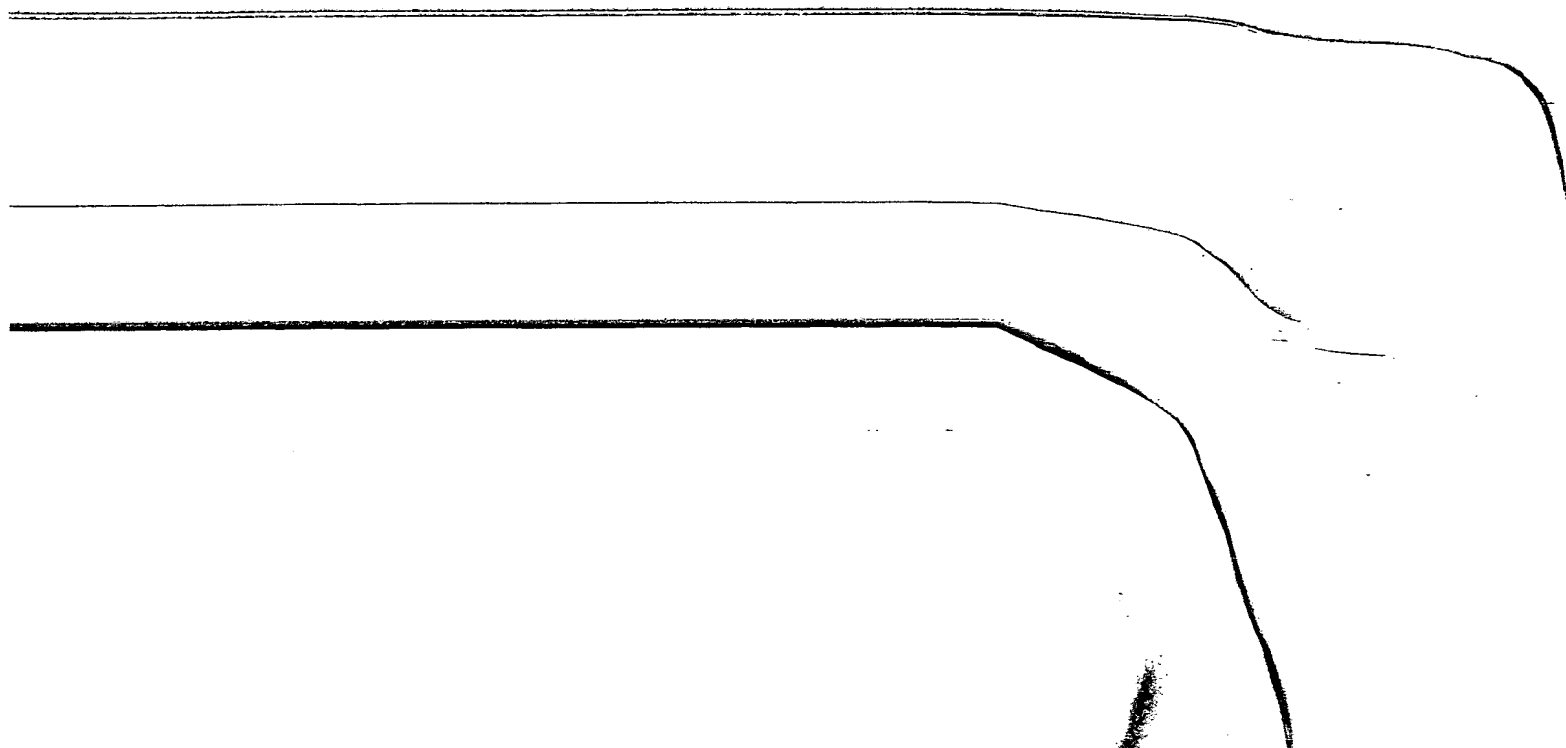
TABLE 2

	<u>Constructs used</u>	<u>observation</u>
10	Sn + C1	red pigmented cells
	Sn only	no pigmented cells
	C1 only	no pigmented cells
	Sn + MYB 75	red pigmented cells
	Sn + MYB90	red pigmented cells
15	Sn + MYB75 + MYB 90	red pigmented cells

These results in Table 2 indicate that AtMYB75 and AtMYB90 are able to complement maize mutants and are able to functional substitute the maize C1 gene in activating the anthocyanin biosynthesis in maize. Thus, anthocyanin was induced in the maize plants. This experiment shows that MYB75 and MYB90 genes cure the defect of a lack of anthocyanin in mutant plants. The experiment also show that the MYB genes may be stably transformed into plants and that cross species introduction of these genes is successful. Applicants believe that the MYB75 and MYB90 genes activate the anthocyanin pathway.

Conclusion

Although the present invention has been described with respect to exemplary embodiments, there are many other variations of the above-described embodiments which will be apparent to those skilled in the art, even where elements have not



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explicitly been designated as exemplary. It is understood that these modifications are within the teaching of the present invention.

We claim:

1. A compound comprising a nucleic acid molecule comprising a sequence that encodes a plant stress tolerance-related myloblastosis (MYB) transcription factor.
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2. The compound of claim 1, wherein the MYB transcription factor is selected from the group consisting of MYB60, MYB74, MYB75, and MYB90.
3. The compound of claim 1, wherein the nucleic acid molecule encodes
10 an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
4. The compound of claim 1, wherein the nucleic acid molecule encodes an amino acid sequence having a sequence identity of about 50% to about 100%
15 with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
5. The compound of claim 1, wherein the nucleic acid molecule encodes an amino acid sequence having a sequence identity of about 65% to about 99% with
20 an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
6. The compound of claim 1, wherein the nucleic acid molecule encodes an amino acid sequence having a sequence identity of about 70% to about 99% with
25 an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
7. The compound of claim 1, wherein the nucleic acid sequence is a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ
30 ID NO:5, and SEQ ID NO:7.

8. The compound of claim 1, wherein the nucleic acid sequence is a sequence sharing a sequence identity of about 50% to about 100% with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

5

9. The compound of claim 1, wherein the nucleic acid sequence is a sequence sharing a sequence identity of about 65% to about 99% with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

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10. The compound of claim 1, wherein the nucleic acid sequence is a sequence sharing a sequence identity of about 70% to about 99% with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7.

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11. A recombinant vector comprising the nucleic acid molecule of claim 1.

12. A compound comprising a nucleic acid molecule comprising a nucleic acid molecule encoding an RNA molecule which is substantially homologous to at least a portion of an RNA transcript of a plant MYB gene, wherein said plant MYB gene hybridizes under low stringency conditions with a nucleic acid sequence encoding a MYB transcription factor selected from the group consisting of MYB60, MYB74, MYB75, and MYB90.

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13. The compound of claim 12, wherein the MYB transcription factor is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

14. The compound of claim 12, wherein the MYB transcription factor has a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
- 5
15. The compound of claim 12, wherein the MYB transcription factor has a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
- 10
16. The compound of claim 12, wherein the MYB transcription factor has a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
- 15
17. The compound of claim 12, wherein the MYB gene has a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.
- 20
18. The compound of claim 12, wherein the MYB gene has a sequence identity of about 50% to about 100% with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.
- 25
19. The compound of claim 12, wherein the MYB gene has a sequence identity of about 65% to about 99% with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

20. The compound of claim 12, wherein the MYB gene has a sequence identity of about 70% to about 99% with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

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21. The compound of claim 12, wherein the nucleic acid molecule comprises at least about six nucleotides.

22. A recombinant vector for transformation of plant cells, comprising
10 a nucleic acid molecule substantially homologous to (1) at least a portion of a DNA molecule encoding a MYB transcription factor selected from the group consisting of MYB60, MYB74, MYB75 and MYB90, or (2) at least a portion of an RNA sequence encoded by the DNA molecule encoding said MYB transcription factor; and

15 regulatory sequences operatively linked to the nucleic acid molecule such that the nucleic acid molecule is expressed in a plant cell into which it is transformed.

23. A MYB polypeptide comprising a plant stress tolerance-related MYB
20 transcription factor.

24. The MYB polypeptide of claim 23, wherein the MYB polypeptide is a MYB transcription factor having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

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25. The MYB polypeptide of claim 23, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

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26. The MYB polypeptide of claim 23, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

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27. The MYB polypeptide of claim 23, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

10

28. An antibody of the MYB polypeptide of claim 23.

29. The antibody of claim 28, wherein the MYB polypeptide is a MYB transcription factor having a sequence selected from the group consisting of SEQ ID
15 NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

30. The antibody of claim 28, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID
20 NO:4, SEQ ID NO:6, and SEQ ID NO:8.

31. The antibody of claim 28, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID
25 NO:4, SEQ ID NO:6 and SEQ ID NO:8.

32. The antibody of claim 28, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID
30 NO:4, SEQ ID NO:6, and SEQ ID NO:8.

33. A variant of the MYB polypeptide of claim 23.

34. The variant of claim 33, wherein the MYB polypeptide is a MYB transcription factor having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

35. The variant of claim 33, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

36. The variant of claim 33, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

37. The variant of claim 33, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

38. A method for enhancing a plant's tolerance to stress comprising transforming said plant with a vector as claimed in claim 11.

39. The method of claim 38, wherein said plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

40. A method of producing a transgenic plant with enhanced stress tolerance comprising:

transforming at least one plant cell with a recombinant DNA construct comprising a nucleic acid sequence encoding a MYB transcription factor; and
regenerating a transgenic plant from the transformed cell, whereby the increased expression of the MYB transcription factor confers enhanced stress
5 tolerance to the plant.

41. The method of claim 40, wherein said MYB transcription factor is selected from the group consisting of MYB60, MYB74, MYB75 and MYB90.

10 42. The method of claim 40, wherein said MYB transcription factor has a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

43. The method of claim 40, wherein the MYB transcription factor shares
15 a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

44. The method of claim 40, wherein the MYB transcription factor shares
20 a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

45. The method of claim 40, wherein the MYB transcription factor shares
25 a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

46. The method of claim 40, wherein the nucleic acid sequence encoding
30 a MYB transcription factor is operatively linked to a promoter.

47. The method of claim 40, wherein the stress tolerance comprises salt stress tolerance.

48. The method of claim 40, wherein the stress tolerance comprises
5 drought stress tolerance.

49. The method of claim 40, wherein the stress tolerance comprises cold stress tolerance.

10 50. The method of claim 40, wherein the stress tolerance comprises heat stress tolerance.

51. The method of claim 40, wherein the nucleic acid sequence is a DNA sequence.
15

52. The method of claim 40, wherein the nucleic acid sequence is an RNA sequence.

52. The method of claim 40, wherein the nucleic acid sequence is a single
20 stranded sequence.

52. The method of claim 40, wherein the nucleic acid sequence is a double stranded sequence.

25 53. The method of claim 40, wherein said plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

54. A method of increasing the expression of a MYB transcription factor in a plant comprising:

- transforming at least one plant cell with a recombinant DNA construct comprising a nucleic acid sequence encoding a MYB transcription factor; and
regenerating a transgenic plant from the transformed cell, wherein the expression of the MYB transcription factor is increased relative to a non-
5 transformed plant and whereby the increased expression of the MYB transcription factor confers enhanced stress tolerance to the plant.

10 55. The method of claim 54, wherein said MYB transcription factor is selected from the group consisting of MYB60, MYB74, MYB75 and MYB90.

56. The method of claim 54, wherein said MYB transcription factor has a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

15 57. The method of claim 54, wherein the MYB transcription factor shares a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

20 58. The method of claim 54, wherein the MYB transcription factor shares a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO 8.

25 59. The method of claim 54, wherein the MYB transcription factor shares a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

60. The method of claim 54, wherein the increased expression of the MYB transcription factor increases the stress tolerance of the plant.

5 61. The method of claim 60, wherein the stress tolerance comprises salt stress tolerance.

62. The method of claim 60, wherein the stress tolerance comprises drought stress tolerance.

10 63. The method of claim 60, wherein the stress tolerance comprises cold stress tolerance.

64. The method of claim 60, wherein the stress tolerance comprises heat stress tolerance.
15

65. The method of claim 54, wherein the nucleic acid sequence is a DNA sequence.

66. The method of claim 54, wherein the nucleic acid sequence is an RNA sequence.
20

67. The method of claim 54, wherein the nucleic acid sequence is a single stranded sequence.
25

68. The method of claim 54, wherein the nucleic acid sequence is a double stranded sequence.

69. The method of claim 54, wherein said plant is selected from the group consisting of whole plants, plant parts and progeny thereof.
30

70. A method of increasing the stress tolerance of a plant comprising:
transforming at least one plant cell with a recombinant DNA construct
comprising a nucleic acid sequence encoding a MYB transcription factor; and
regenerating a transgenic plant from the transformed cell, wherein the
5 expression of the MYB transcription factor is increased relative to a non-
transformed plant and whereby the increased expression of the MYB transcription
factor confers enhanced stress tolerance to the plant.

71. The method of claim 70, wherein said MYB transcription factor is
10 selected from the group consisting of MYB60, MYB74, MYB75 and MYB90.

72. The method of claim 70, wherein said MYB transcription factor has a
sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ
ID NO:6, and SEQ ID NO:8.
15

73. The method of claim 70, wherein the MYB transcription factor shares
a sequence identity of about 50% to about 100% with an amino acid sequence
selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,
and SEQ ID NO:8.
20

74. The method of claim 70, wherein the MYB transcription factor shares
a sequence identity of about 65% to about 99% with an amino acid sequence
selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,
and SEQ ID NO:8.
25

75. The method of claim 70, wherein the MYB transcription factor shares
a sequence identity of about 70% to about 99% with an amino acid sequence
selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,
and SEQ ID NO:8.
30

76. The method of claim 70, wherein the stress tolerance comprises salt stress tolerance.

5 77. The method of claim 70, wherein the stress tolerance comprises drought stress tolerance.

78. The method of claim 70, wherein the stress tolerance comprises cold stress tolerance.

10

79. The method of claim 70, wherein the stress tolerance comprises heat stress tolerance.

80. The method of claim 70, wherein the nucleic acid sequence is a DNA
15 sequence.

81. The method of claim 70, wherein the nucleic acid sequence is an RNA sequence.

20 82. The method of claim 70, wherein the nucleic acid sequence is a single stranded sequence.

83. The method of claim 70, wherein the nucleic acid sequence is a double stranded sequence.

25

84. The method of claim 70, wherein said plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

85. A method for enhancing a plant's sensitivity to stress comprising transforming said plant with a vector as claimed in claim 22.

86. The method of claim 85, wherein said plant is selected from the
5 group consisting of whole plants, plant parts and progeny thereof.

87. A method of producing a stress sensitive transgenic plant having a reduced level of MYB transcription factors comprising:

transforming a plant with a vector comprising an antisense nucleic acid
10 molecule substantially complementary to at least a portion of a DNA molecule encoding a MYB transcription factor or at least a portion of an RNA sequence encoded by the DNA molecule encoding said MYB transcription factor; and regulatory sequences operatively linked to the antisense nucleic acid molecule;
allowing the plant to grow to at least a plantlet stage;
15 assaying the transformed plant or plantlet for altered MYB activity and/or environmental stress sensitivity; and
selecting and growing a plant having altered MYB activity and/or environmental stress sensitivity compared to a non-transformed plant.

88. The method of claim 87, wherein said MYB transcription factor is
20 selected from the group consisting of MYB60, MYB74, MYB75 and MYB90.

89. The method of claim 87, wherein said MYB transcription factor has a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ
25 ID NO:6, and SEQ ID NO:8.

90. The method of claim 87, wherein said MYB transcription factor shares a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,
30 and SEQ ID NO:8.

91. The method of claim 87, wherein said MYB transcription factor shares a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

5

92. The method of claim 87, wherein said MYB transcription factor shares a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

10

93. The method of claim 87, wherein said plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

15

94. The method of claim 87, wherein said plant acts as an environmental monitor.

95. A transgenic plant produced by the transformation of at least one cell of a plant with the recombinant vector of claim 11.

20

96. The transgenic plant of claim 95, wherein the plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

97. A transgenic plant produced by the transformation of at least one cell of a plant with the recombinant vector of claim 22.

25

98. The transgenic plant of claim 97, wherein the plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

99. A seed produced by the transformation of at least one cell of a seed, plant, plant part or progeny thereof with the recombinant vector of claim 11.

100. A seed produced by the transformation of at least one cell of a seed,
5 plant, plant part or progeny thereof with the recombinant vector of claim 22.

101. A method of screening a plant for stress tolerance comprising screening the expression level of a stress tolerance-related MYB transcription factor in a plant.

10

102. The method of claim 101, wherein the plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

103. A transgenic plant stably transformed with a nucleic acid molecule
15 comprising a MYB gene, which is expressed so as to enhance stress tolerance of said plant.

104. The transgenic plant of claim 103, wherein the plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

20

105. The transgenic plant of claim 103, wherein said nucleic acid molecule further comprises a screenable marker gene.

106. A transgenic plant stably transformed with a nucleic acid molecule
25 that encodes an RNA molecule which is substantially homologous to at least a portion of an RNA transcript of a plant MYB gene, wherein said plant MYB gene hybridizes under low stringency conditions with a nucleic acid sequence encoding a MYB transcription factor selected from the group consisting of MYB60, MYB74, MYB75 and MYB90, and wherein said DNA molecule is expressed so as to enhance
30 stress sensitivity of said plant.

107. The transgenic plant of claim 106, wherein the plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

5 108. The transgenic plant of claim 106, wherein said nucleic acid molecule further comprises a screenable marker gene.

109. An isolated nucleic acid molecule comprising a sequence that encodes a plant stress tolerance-related MYB transcription factor, wherein said
10 MYB transcription factor comprises a MYB transcription factor selected from the group consisting of MYB60, MYB74, MYB75 and MYB90.

110. The isolated nucleic acid molecule of claim 109, wherein the DNA molecule hybridizes under low stringency conditions with a nucleic acid sequence
15 selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7 or a variant of the isolated nucleic acid molecule.

111. The isolated nucleic acid molecule of claim 109, wherein the DNA molecule hybridizes under low stringency conditions with a nucleic acid sequence
20 encoding an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8 or a variant of the isolated nucleic acid molecule.

112. An isolated nucleic acid molecule which encodes an RNA molecule
25 which is substantially homologous to at least a portion of an RNA transcript of a plant MYB gene, wherein said plant MYB gene hybridizes under low stringency conditions with a nucleic acid sequence encoding plant stress sensitivity-related MYB transcription factor selected from the group consisting of MYB60, MYB74, MYB75 and MYB90.

30

113. The isolated nucleic acid molecule of claim 112, wherein the said plant MYB gene hybridizes under low stringency conditions with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7 or a variant of the isolated nucleic acid molecule.

5

114. The isolated nucleic acid molecule of claim 112, wherein the said plant MYB gene hybridizes under low stringency conditions with a nucleic acid sequence encoding a plant stress sensitivity-related MYB transcription factor having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8 or a variant of the isolated nucleic acid molecule.

115. A method for increasing the stress resistance of a crop in a field comprising planting in the field seeds or plants comprising transgenic plants or seeds transformed with the vector of claim 11.

15

116. A method of inhibiting the expression of MYB genes in a plant cell, said method comprising:

(1) integrating into the genome of a plant a vector comprising (a) an antisense nucleic acid molecule substantially complementary to (i) at least a portion of a DNA molecule encoding a MYB transcription factor selected from the group consisting of MYB60, MYB74, MYB75 and MYB90, or (ii) at least a portion of an RNA sequence encoded by the DNA molecule encoding said MYB transcription factor; and (b) regulatory sequences operatively linked to the antisense nucleic acid molecule such that the nucleic acid molecule is expressed in a plant cell into which it is transformed; and

20

(2) growing said plant, whereby said antisense nucleic acid molecule is transcribed, whereby expression of said MYB gene is inhibited.

25

117. A method of assaying environmental conditions of a field comprising planting at least one plant as in claim 103 in a field; and monitoring for growth of said plant.
- 5 118. A method of assaying environmental conditions of a field comprising planting at least one plant as in claim 106 in a field; and assaying for growth of said plant.
- 10 119. A method of increasing the production of products of the phenylpropanoid biosynthesis pathway in a plant comprising:
transforming a plant cell with a recombinant DNA construct comprising a nucleic acid sequence encoding a MYB transcription factor; and
regenerating a transgenic plant from the transformed cell, wherein the expression of the MYB transcription factor increases the expression of genes
15 encoding gene products affecting the phenylpropanoid pathway, thereby increasing the production of products of the phenylpropanoid biosynthesis pathway.
- 20 120. The method of claim 119, wherein the products of the phenylpropanoid pathway include one or more of stilbenes, flavonoids, lignins, salicylic acid, anthocyanins, and phenolic derivatives.

AtMYB60

- cDNA = 949 bp
- Peptide = 281 aa

Nucleic acid
SEQ ID NO 1

Amino acid
SEQ ID NO 2

```

1      GAGAGAGAAAGATGGGTAGGCCTCCATGCTGTGACAAGATAGGGATCAAGAAAGGACCAT
      M G R P P C C D K I G I K K G P W
61     GGACTCCTGAAGAAGATATCATTCTTGTCTTACATTCAAGAACATGGTCCTGGAAACT
      T P E E D I I L V S Y I Q E H G P G N W
121    GGAGATCAGTTCCCAACAACTGGGTTATTGAGATGCAGCAAAAGTTGTAGACTGAGAT
      R S V P T N T G L L R C S K S C R L R W
181    GGACAAATTATCTGAGACCTGGAATTAAACGTGGAACTTTACTCCTCATGAAGAAGGAA
      T N Y L R P G I K R G N F T P H E E G M
241    TGATCATTCACTTGCAAGCCTTATGGGTAACAAATGGGCGTCCATAGCTTCATACCTAC
      I I H L O A L L G N K W A S I A S Y L P
301    CACAAAGAACGGACAATGATATCAAGAACTACTGGAACACACATTTAAAGAAGAAGCTCA
      Q R T D N D I K N Y W N T H L K K K L N
      MYB60FII
361    ACAAGTCTGACAGTGATGAGAGGAGCAGATCAGAGAACATTGCGCTGCAAACTTCTTGA
      K S D S D E R S R S E N I A L Q T S S T
421    CAAGAAACACCATTAATCATAGATCTACCTATGCTTCAAGCACAGAAAACATTTCCCGCC
      R N T I N H R S T Y A S S T E N I S R L
481    TTGTGGAGGGTTGGATGAGAGCGTCTCCAAAGAGTAGTACAAGTACTACTTTCTTGGAAC
      V E G W M R A S P K S S T S T T F L E H
541    ACAAATGCAGAACCGGACAACAATTTTCATCGATCATCACAGTGATCAGTTTCCATACG
      K M Q N R T N N F I D H H S D Q P P Y E
601    AGCAGCTTCAAGGTTCTAGGGAAGAGGGTCATAGCAAAGGAATCAACGGGGATGATGACC
      Q L Q G S R E E G H S K G I N G D D D Q
661    AGGGTATAAAGAATTCAGAGAATAACAACGGTGATGATGTTTCATCATGAAGATGGTGATC
      G I K N S E N N N G D D V H H E D G D H
721    ATGAGGATGATGATGATCATAATGCAACACCACCATTGACATTTATTGAGAAATGGCTTT
      E D D D D H N A T P P L T F I E K W L L
      MYB60RIII
781    TGGAGGAAACAAGTACTACTGGGGGTCAAATGGAAGAGATGAGGAGGATGATGAGGAGGAT
      E E T S T T G G Q M E E M S H L M E L S
841    ATGAGGATGATGATGATCATAATGCAACACCACCATTGACATTTATTGAGAAATGGCTTT
      N M L *
901    TAAATGAGACTACTAATTTTATATACACAAATAAAGAAACCAGAAAGAC
  
```

Fig 1

AtMYB74

- cDNA = 901 bp
- Peptide = 260 aa

Nucleic acid
SEQ ID No: 3

amino acid
SEQ ID No: 4

1 TATTAAGCGTGAAGATTCTCTTTTGAAGAAGAAGAAACCATTATTCAACTTCACGGCAT
I K R G R F S F E E E E T I I Q L H G I

61 CATGGGAAACAAGTGGTCTGCGATTGCGGCTCGTTTGCCTGGAAGAACAGACAACGAGAT
M G N K W S A I A A R L P G R T D N E I
MYB74FII

121 CAAAACTATTGGAACACTCACATCAGAAAAAGACTTCTAAAGATGGGAATCGACCCGGT
K N Y W N T H I R K R L L K M G I D P V

181 TACACACACTCCACGTCTTGATCTTCTCGATATCTCCTCCATTCTCAGCTCATCTATCTA
T H T P R L D L L D I S S I L S S I Y

241 CAACTCTTCGCATCATCATCATCATCATCAACAACATATGAACATGTGAGGGCTCAT
N S S H H H H H H H Q Q H M N M S R L M

301 GATGAGTGATGGTAATCATCAACCATTGGTTAACCCCGAGATACTCAAACCTCGCAACCTC
M S D G N H Q P L V N P E I L K L A T S

361 TCTCTTTTCAAACCAAACACCCCAACAaCACACACGAGAACAACACGGTTAACCAAAC
L F S N Q N H P N N T H E N N T V N Q T

421 CGAAGTAAACCAATACCAAACCGGTTACAACATGCCTGGTAATGAAGAATTACAATCTTG
E V N Q Y Q T G Y N M P G N E E L Q S W

481 GTTCCCTATCATGGATCAATTCACGAATTTCCAAGACCTCATGCCAATGAAGACGACGGT
F P I M D Q F T N F Q D L M P M K T T V

541 CCAAATTCATTGTCATACGATGATGATTGTTTGAAGTCCAATTTGTATTAGAACCTTA
Q N S L S Y D D D C S K S N F V L E P Y

601 TTACTCCGACTTTGCTTCAGTCTTGACCACACCTTCTTCAAGCCCGACTCCGTTAAACTC
Y S D F A S V L T T P S S S P T P L N S
MYB74RIII

661 AAGTTCCTCAACTTACATCAATAGTAAGTTCACGACCAAGAGTAAAGAGAGTTA
S S S T Y I N S S T C S T E D E K E S Y

721 TTACAGTGATAATATCACTAATTATTCGTTTGATGTTAATGGTTTTCTCCAATTCCAATA
Y S D N I T N Y S F D V N G F L Q P Q *

781 AACAAACGCCATTGGAATAGAGTTATGTAAACATGCAATCATTTGTATTGTATATAGA
841 TTTTGTACATATCCAAATCCAAATACTATAGTTTTAAATAAAAAAAAAAAAAAAAAA
901 A

Fig²

AtMYB 75 cDNA sequence

SEQ ID No. 5

CCACGCGTCCGTACCTTTTACAATTTGTTTATATATTTTACGTATCTATCTTTGTTCCATGGA
GGGTTTCGTCCAAAGGGCTGCGAAAAGGTGCTTGGACTACTGAAGAAGATAGTCTCTTGAG
ACAGTGCATTAATAAGTATGGAGAAGGCAAATGGCACCAAGTTCCTGTAAGAGCTGGGCT
AAACCGGTGCAGGAAAAGTTGTAGATTAAAGATGGTTGAACTATTTGAAGCCAAGTATCAA
GAGAGGAAAACCTTAGCTCTGATGAAGTCGATCTTCTTCTTCGCCTTCATAGGCTTCTAGGG
AATAGGTGGTCTTTAATTGCTGGAAGATTACCTGGTCGGACCGCAAATGAOGTCAAGAATT
ACTGGAACACTCATCTGAGTAAGAAACATGAACCGTGTGTAAGATAAAGATGAAAAAGA
GAGACATTACGCCCATTCCTACAACACCGGCACTAAAAACAATGTTTATAAGCCTCGACC
TCGATCCTTCACAGTTAACAACGACTGCAACCATCTCAATGCCCCACCAAAGTTGACGTT
AATCCTCCATGCCTTGGACTTAACATCAATAATGTTTGTGACAATAGTATCATATACAACA
AAGATAAGAAGAAA GACCAACTAGTGAATAATTGATTGATGGAGATAATATGTGGTTAG
AGAAATTCCTAAGGAAAGCCAAGAGGTAGATATTTTGGTTCCTGAAGCGACGACAACAGA
AAAGGGGGACACCTTGGCTTTTGACGTTGATCAACTTTGGAGTCTTTTCGATGGAGAGACT
GTGAAATTTGATTAGTGTTCGAACATTTGTTTGCCTTTGTGTATAGGTTTGCTTTACCTTT
TAATTTGTGTGTTTTGATAAATAAGCTAATAGTTTTAGCATTTTAATGAAATATTTCAAGT
TTCCGTGTTAC

Fig 3a

Aminoacid sequence of AtMYB 75

MEGSSKGLRKGA WTTTEEDSLLRQCINKY GEGKWHQVPVRAGLNRCRKSCRLRWLNLYLKPSIK
RGKLSSDEV D L L L R L H R L L G N R W S L I A G R L P G R T A N D V K N Y W N T H L S K K H E P C C K I K M K K R D
I T P I T T P A L K N N V Y K P R K S F T V N N D C N H L N A P K Y D V N P P E L G L N N N V G D N S I P N K D K K K
D Q L V N N L I D G D N M W L E K F L R K A K R

Fig 3b

SEQ ID No. 6

MYB90

cDNA = 1043 bp
Peptide = 250 aa

Nucleic acid
SEQ ID No: 7

Amino Acid
SEQ ID No: 8

GTGACCCACGCGTCCGTGGGAAGCCACAATAACCCCTATTCTCGGCCTTTTAA
AAGTTTGTAGAATAATCCGATAAAATACCTTTATATTAATTTTCTTGGTCCATGGAGGG
M E G

TTCGTCCAAAGGGTTGAGGAAAGGTGCATGGACTGCTGAAGAAGATAGTCTTTGAGGCT
S S K G L R K G A W T A E E D S L L R L

ATGTATTGATAAGTATGGAGAAGGCAAATGGCATCAAGTTCCTTTGAGAGCTGGGCTAAA
C I D K Y G E G K W H Q V P L R A G L N

TCGATGCAGAAAGAGTTGTAGACTAAGATGGTTGAACTATTTGAAGCCAAGTATCAAGAG
R C R K S C R L R W L N Y L K P S I K R

AGGAAGACTTAGCAATGATGAAGTTGATCTTCTTTCGCTTCATAAGCTTCTAGGAAA
G R L S N D E V D L L L R L H K L L G N

TAGGTGGTCCCTTGCATTCTGGTGGATTGCTGGTGGGACCGCTAATGATGCAAAATTA
R W S L I A G R L P G R T A N D V K N Y

EST193FB

CTGGAACACCCATCTGAGTAAAAAACATGAGTCTTTCGTGTGTGAAGTCTAAAATGAAAA
W N T H L S K K H E S S C C K S K M K K

GAAAAACATTATTTCCCTCCTACACACCGGTCCAAAAAATCGGTGTTTTTAAGCCTCG
K N I I S P P T T P V Q K I G V F K P R

ACCTCGATCCTTCTCTGTTAACAATGGTTGCAGCCATCTCAATGGTCTGCCAGAAGTTGA
P R S F S V N N G C S H L N G L P E V D

TTTAATTCCTTCATGCCTTGGACTCAAGAAAAATAATGTTTGTGAAAATAGTATCACATG
L I P S C L G L K K N N V C E N S I T C

TAACAAAGATGATGAGAAAGATGATTTTGTGAATAATCTAATGAATGGAGATAATATGTG
N K D D E K D D F V N N L M N G D N M W

GTTGGAGAATTTACTGGGGGAAAAACCAAGAAGCTGATGCGATTGTTCTGAAGCGACGAC
L E N L L G E N Q E A D A I V P E A T T

MYB90RIII

AGCTGAACATGGGGCCACTTTGGCGTTTGAAGTTGAGCAACTTTTCACTCTCTTTTCTCT
A E H G A T L A F D V E Q L W S L F D G

ACACCTCTTGAACCTTGATTAGTGTCTTCTCACCCTTGTGTTAAGATTGTGGGTGGCTTTT
E T V E L D *

CTTTCGTATTTTAGTAATGTATTTTTCTGTATGAAGTAAAGAATTTTCAAGCATTTTAAGAA
AAATGGTTATGTTTCTACGTAATAAAAAAACGTTATTTTATAAAAAA
AAAAAAAAAAGGGCGGCCGC

Fig 4

P5CS1

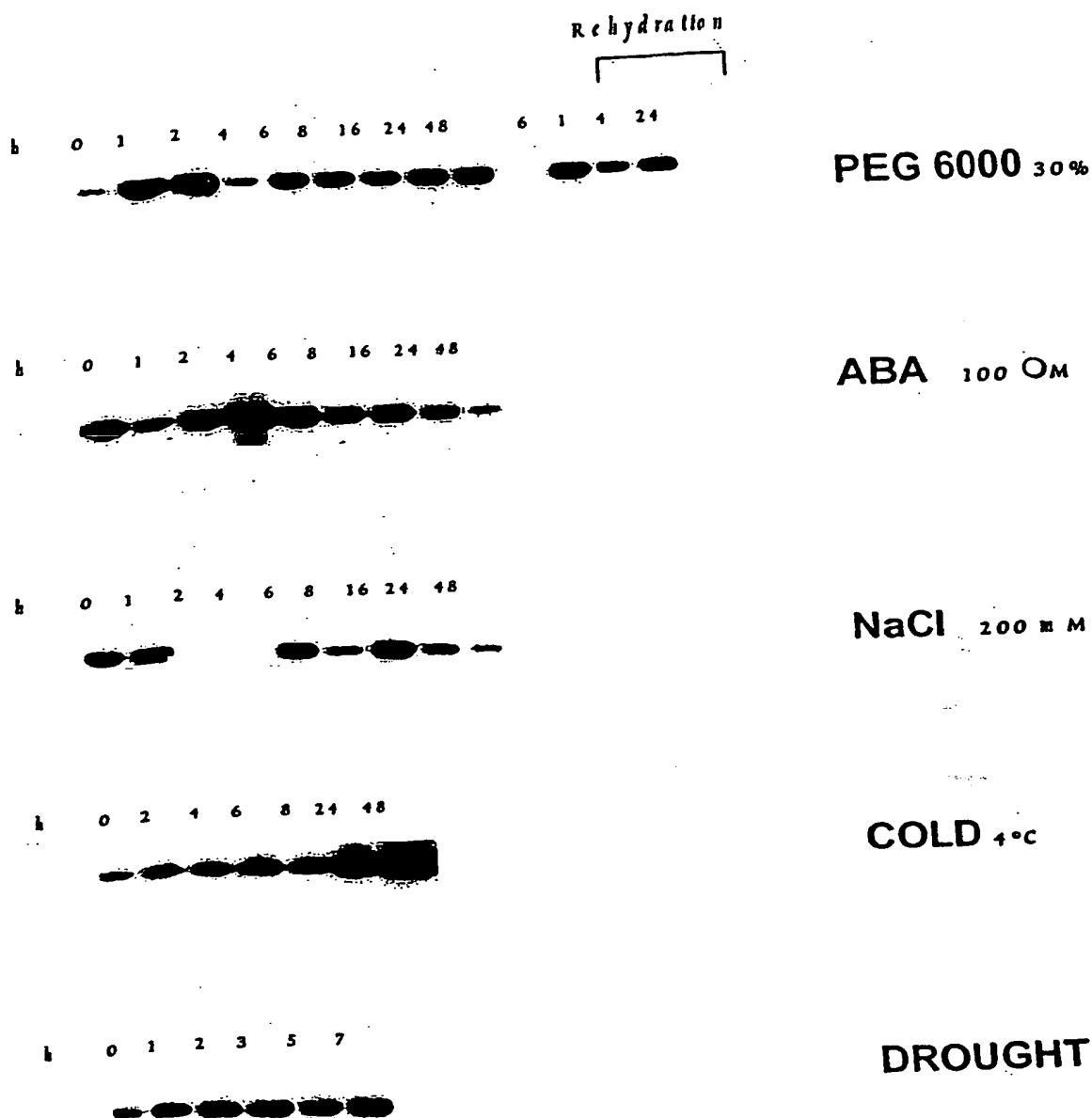


Figure 5. RT-PCR analysis

RD22

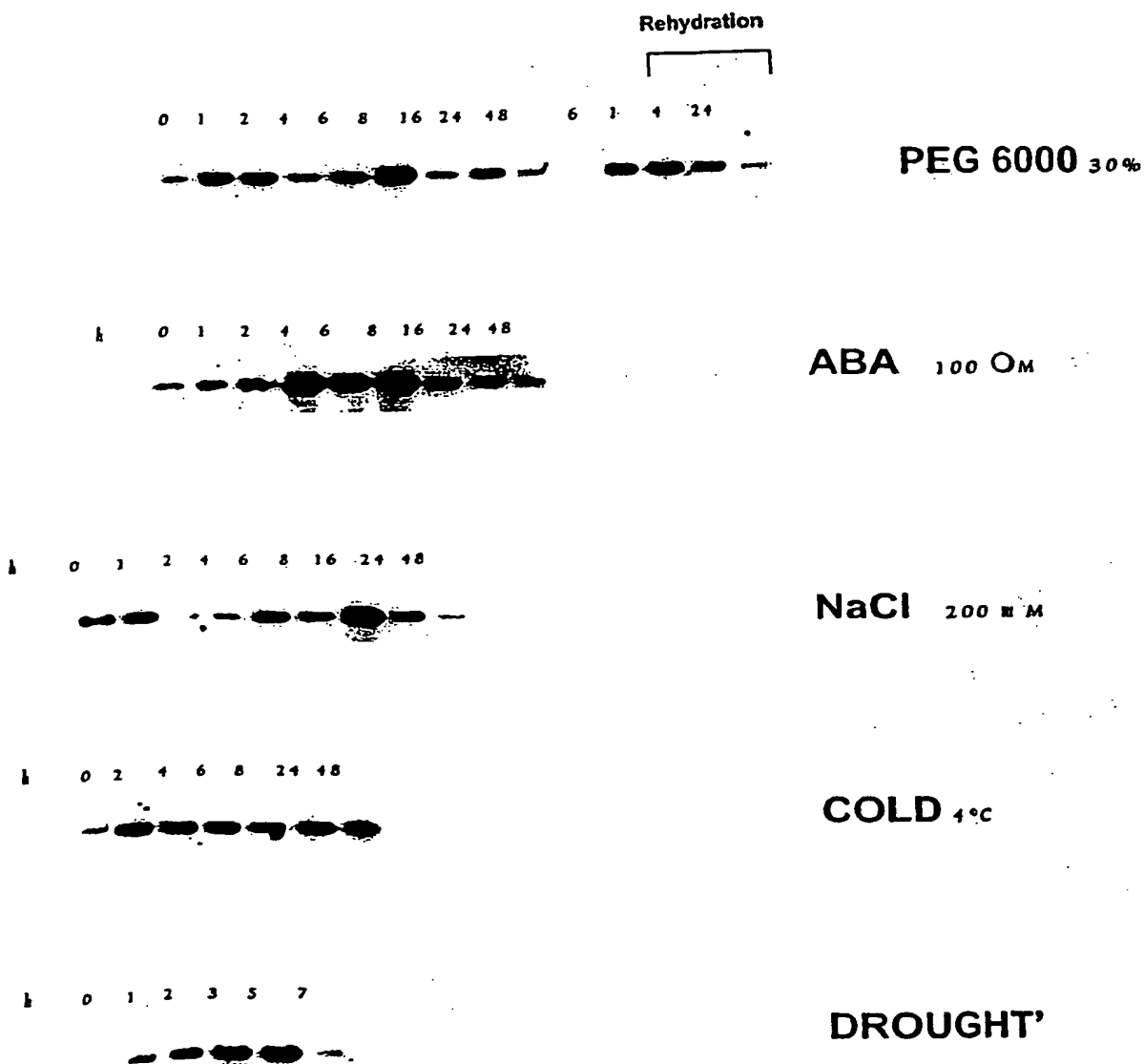


Figure 6. RT-PCR analysis

ERD10

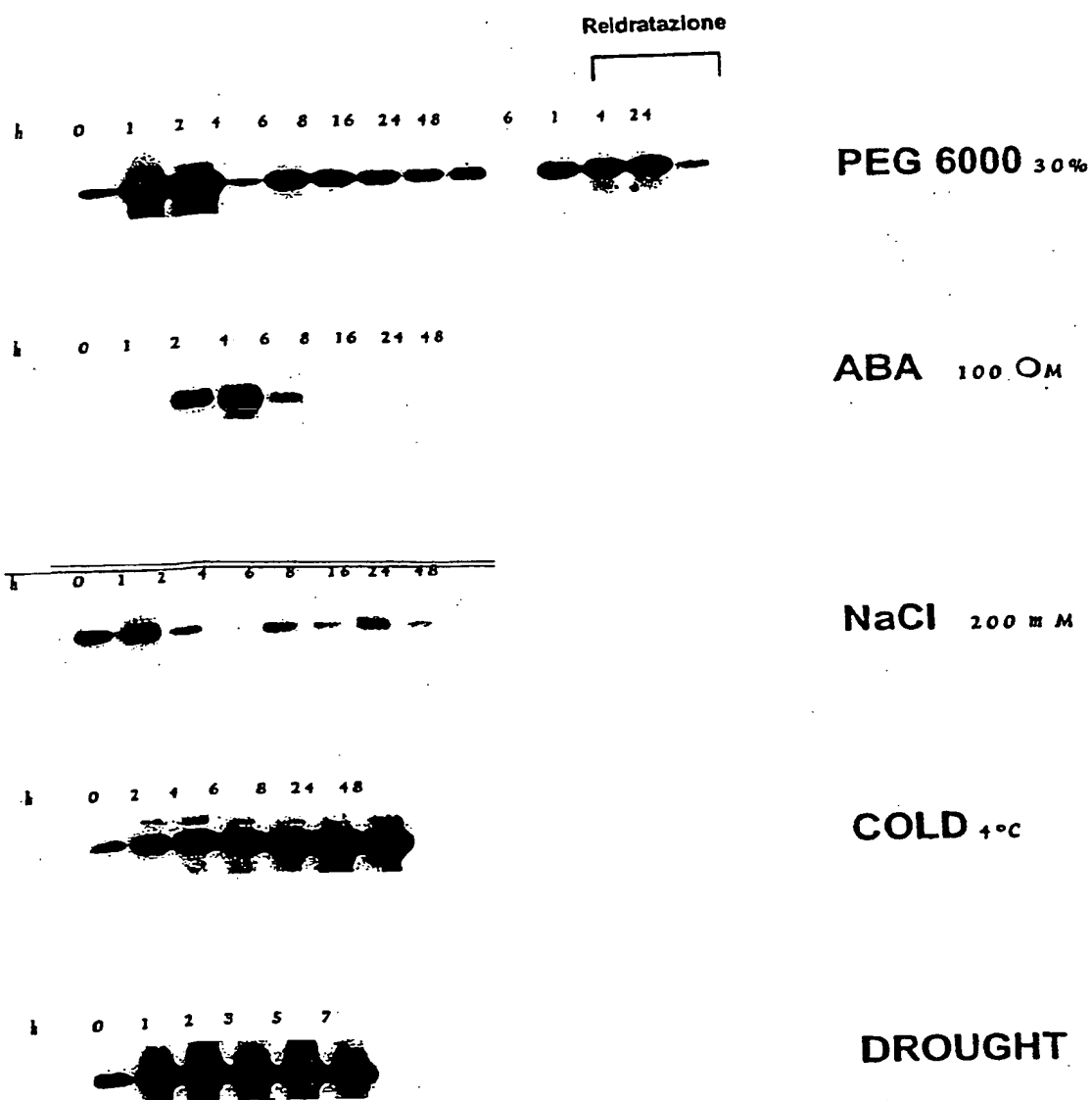


Figure 7. RT-PCR analysis

ADH1

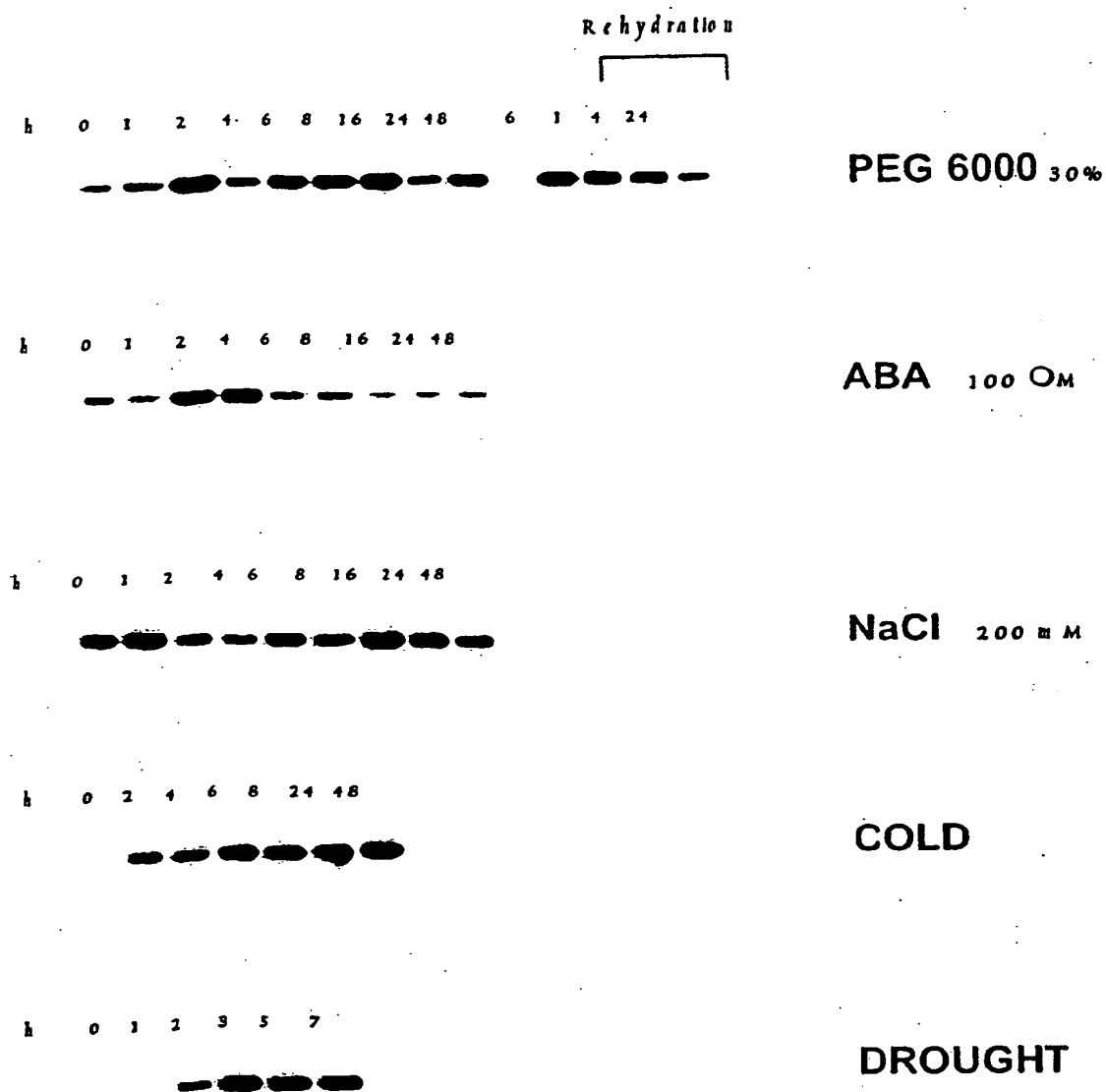


Figure 8. RT-PCR analysis

AtMYB74

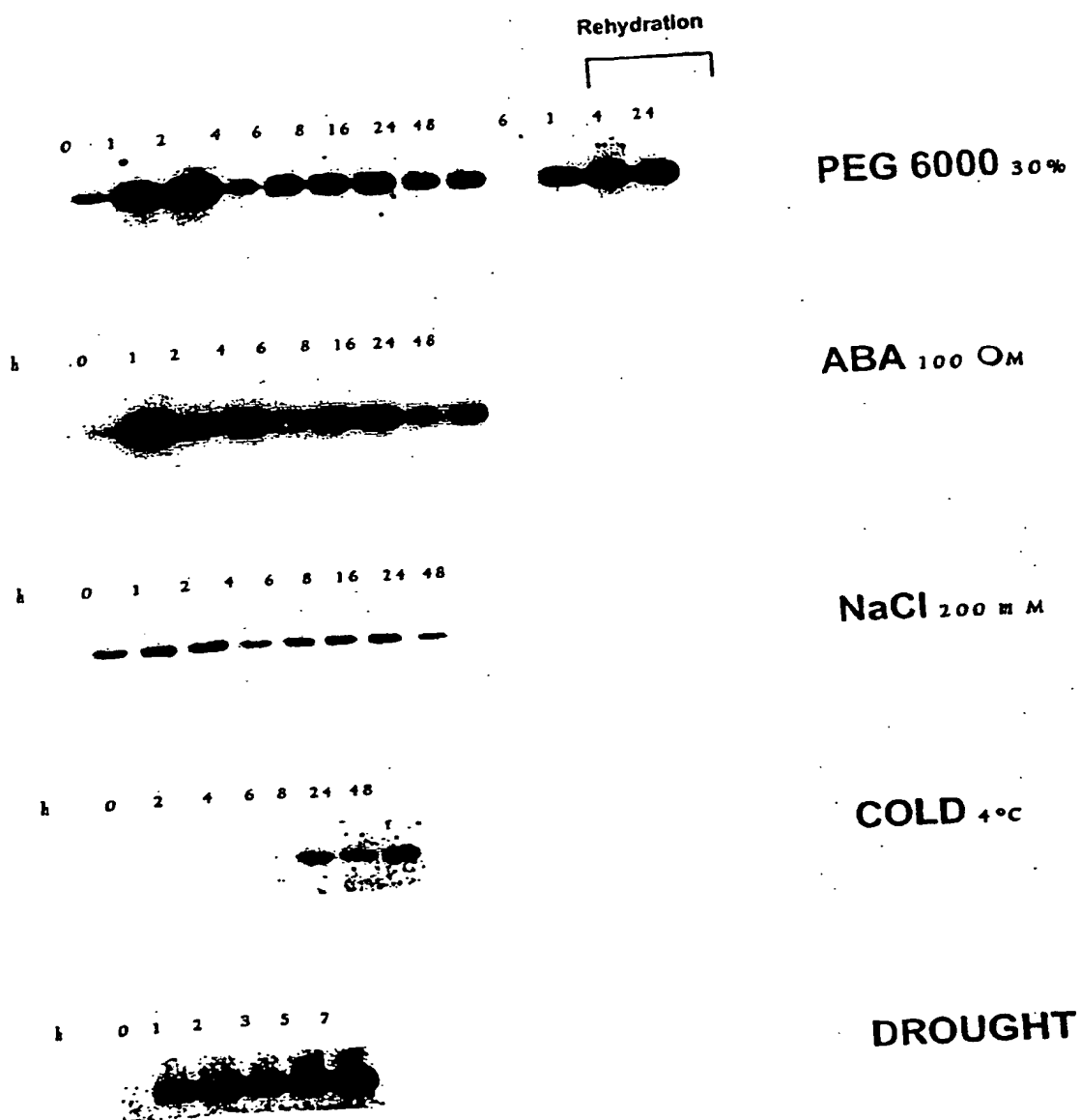


Figure 9. RT-PCR analysis.

AtMYB75

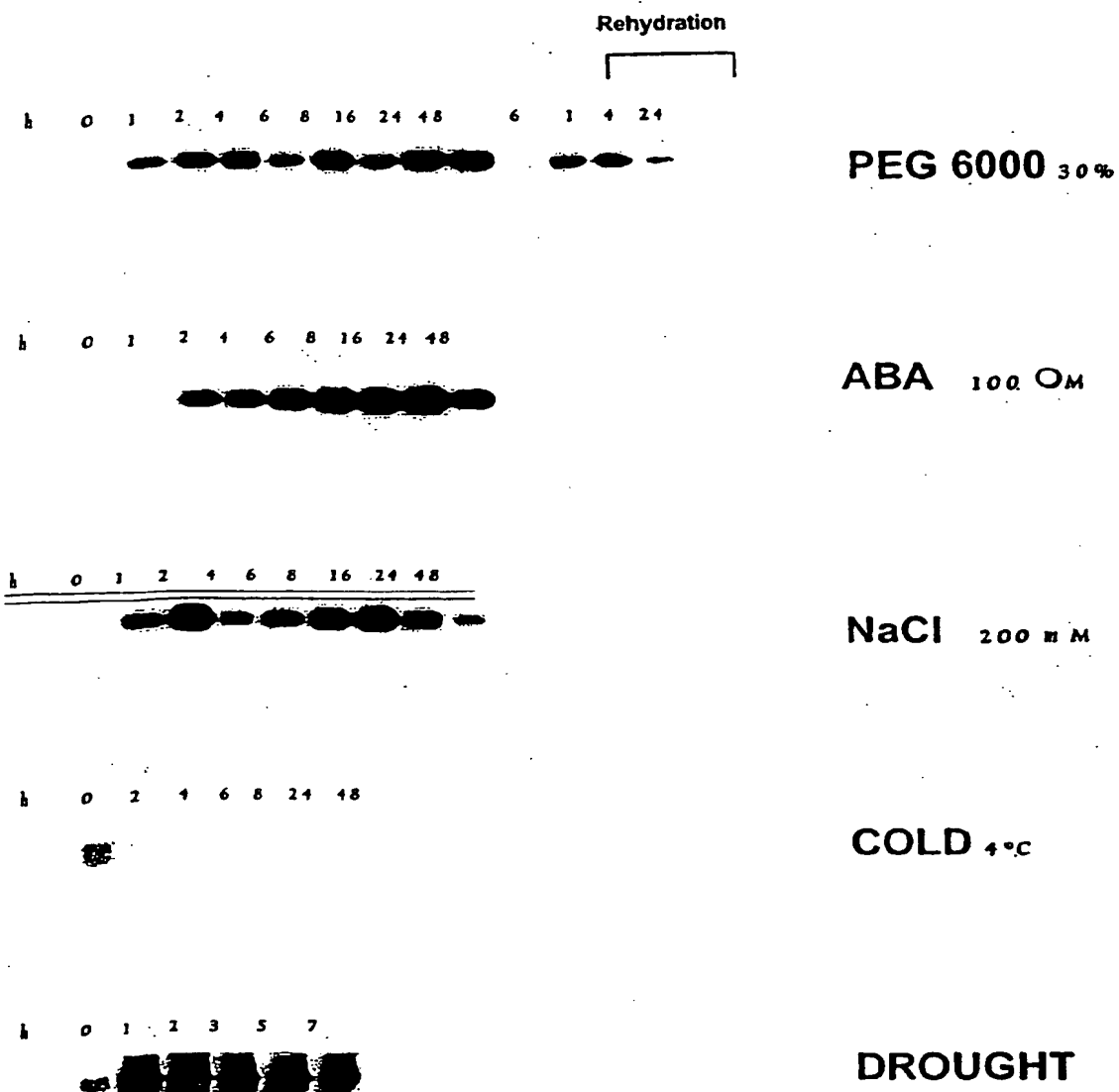


Figure 10. RT-PCR analysis

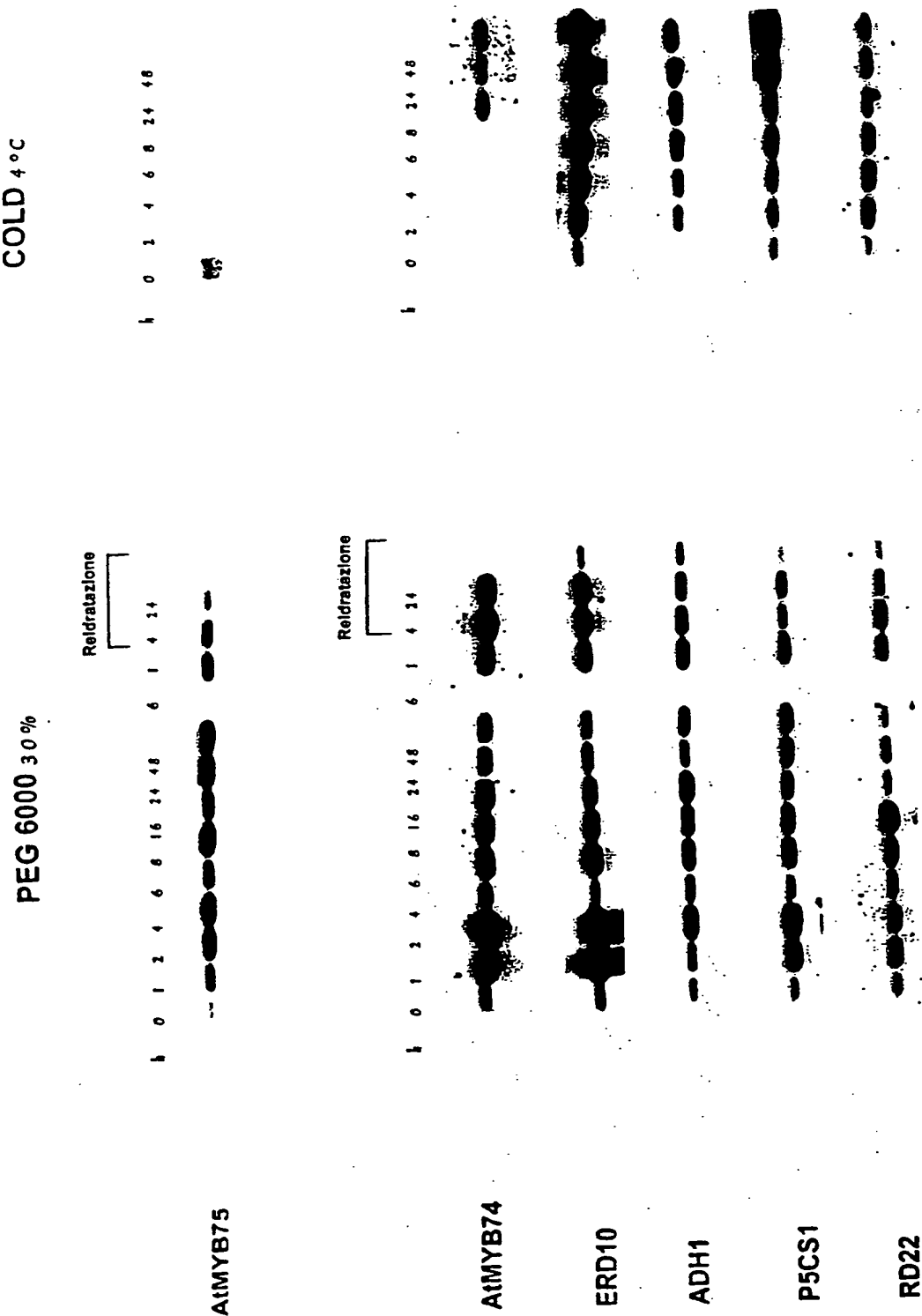


Figura 11: Expression patterns of *AtMYB75*, *AtMYB74*, *ERD10*, *ADH1*, *P5CS1* e *RD22*, following treatments with PEG 30% and COLD 4°C.

AtMYB90

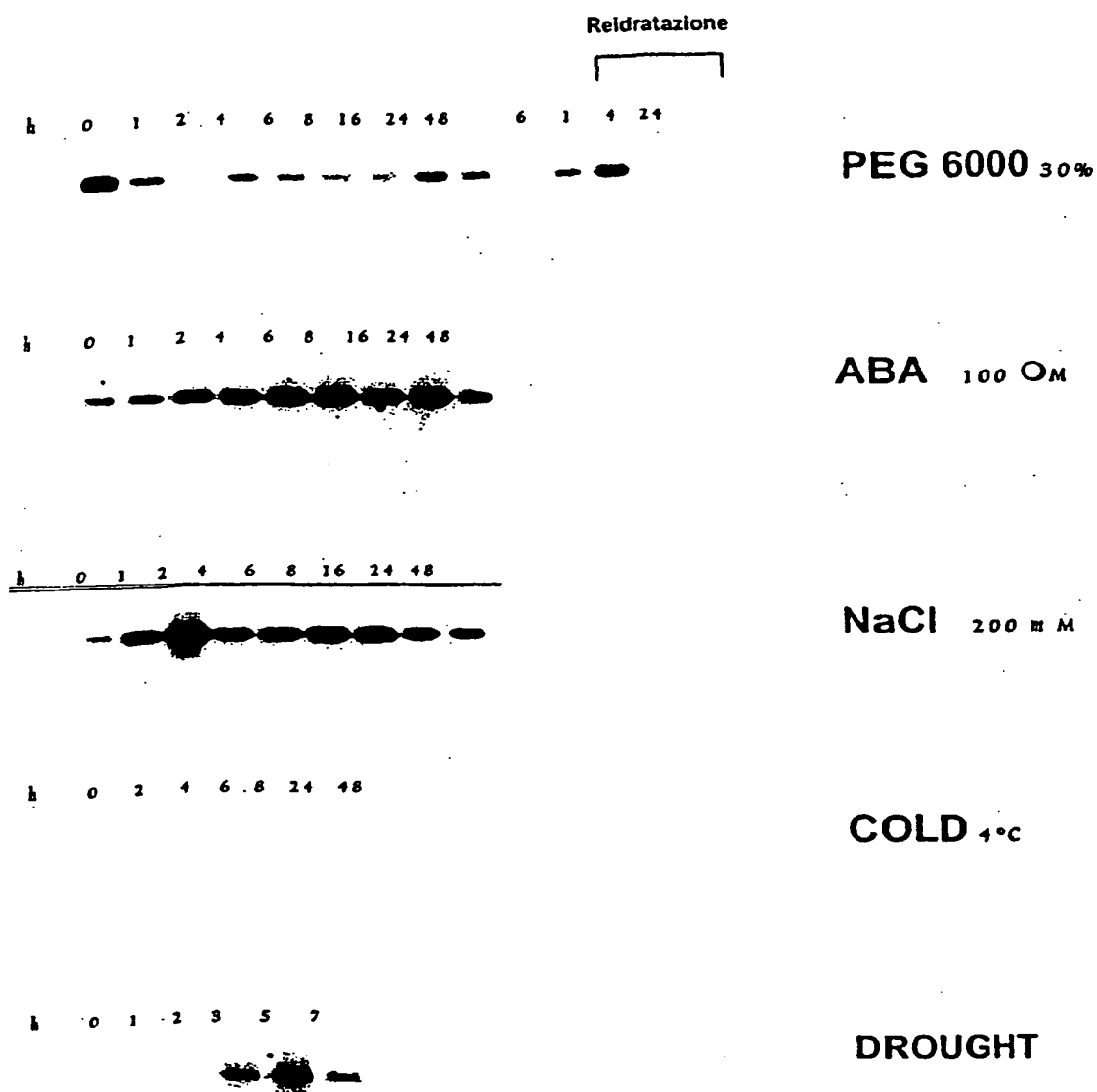


Figure 12. RT-PCR analysis

AtMYB 60

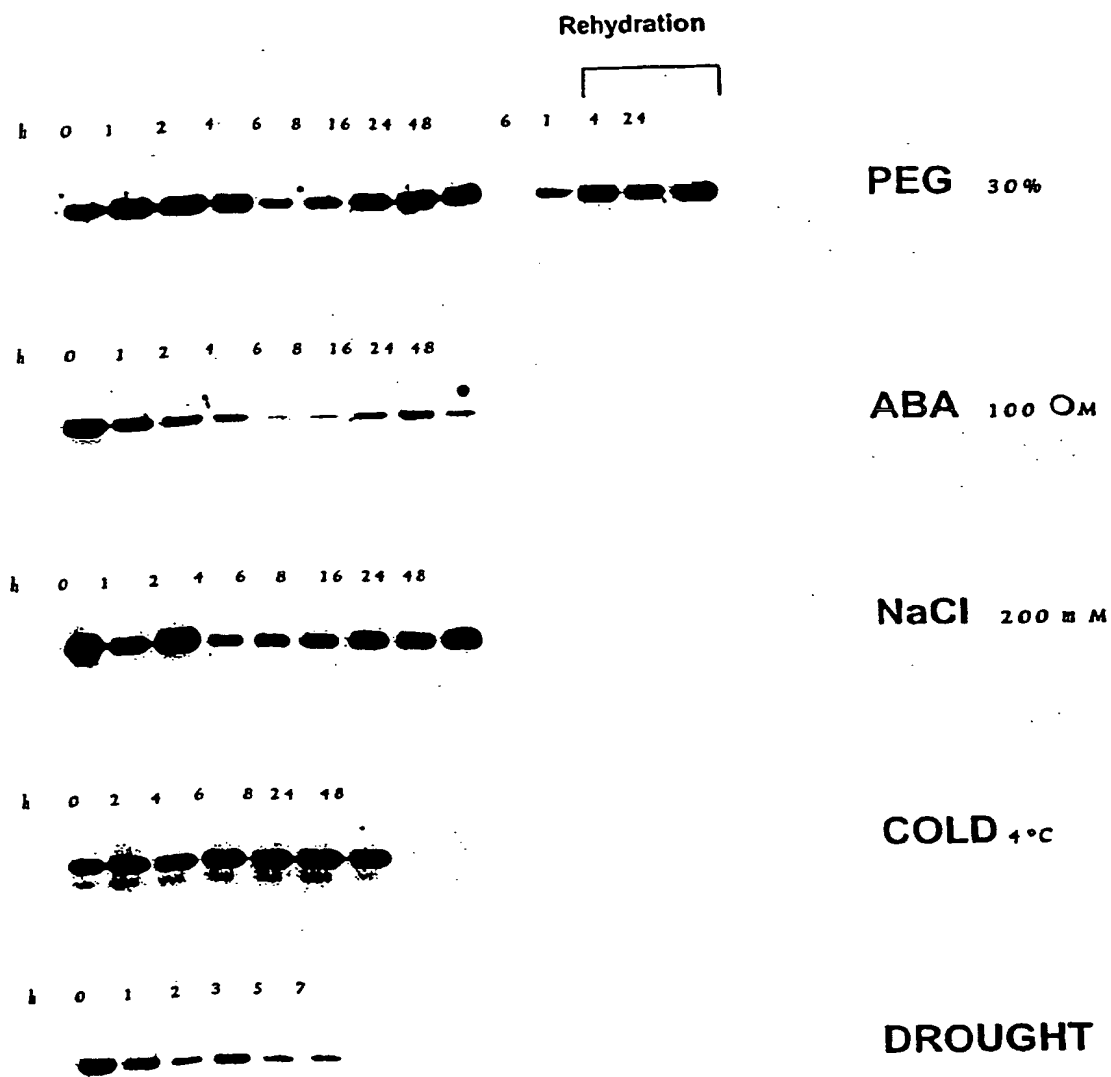


Figure 13. RT-PCR analysis

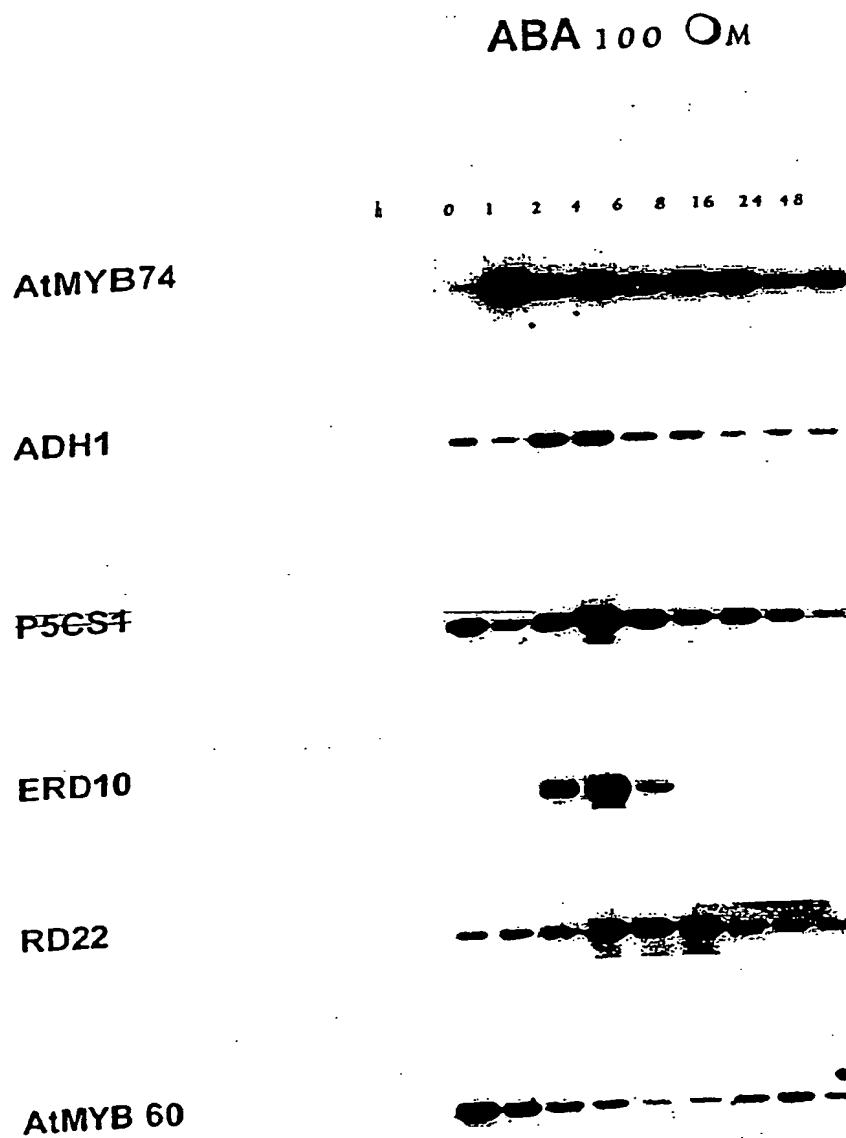


Figure 14: Expression pattern of *AtMYB74*, *ADH1*, *P5CS1*, *ERD10*, *RD22* e *AtMYB60*, following treatments with ABA 100 μ M.

FIGURE 15

COMPARISON BETWEEN THE EXPRESSION OF MYB-75 AND MYB-90 GENES AND STRUCTURAL GENES OF PHENYLPROPANOID PATHWAY AFTER LIGHT TREATMENTS

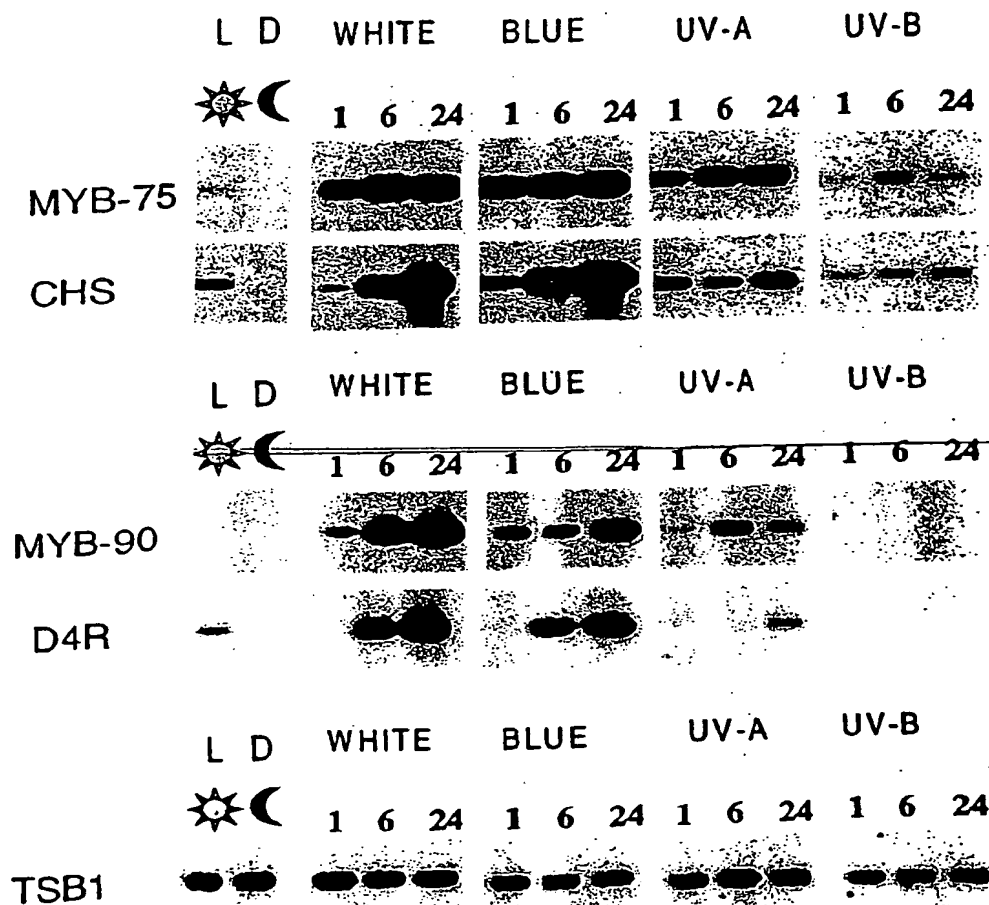


Figure 15. MYB-75 and MYB-90 expression patterns in response to white, blue, UV-A and UV-B light are consistent with their putative role in the control of phenylpropanoid pathway: MYB-75 for the upper part where chalcone synthase (CHS gene) represents the key enzyme and MYB-90 for the lower one where dihydroflavonol-4-reductase (D4R gene) is required (Kubasek et al., 1992; Pelletier and Shirley, 1996).

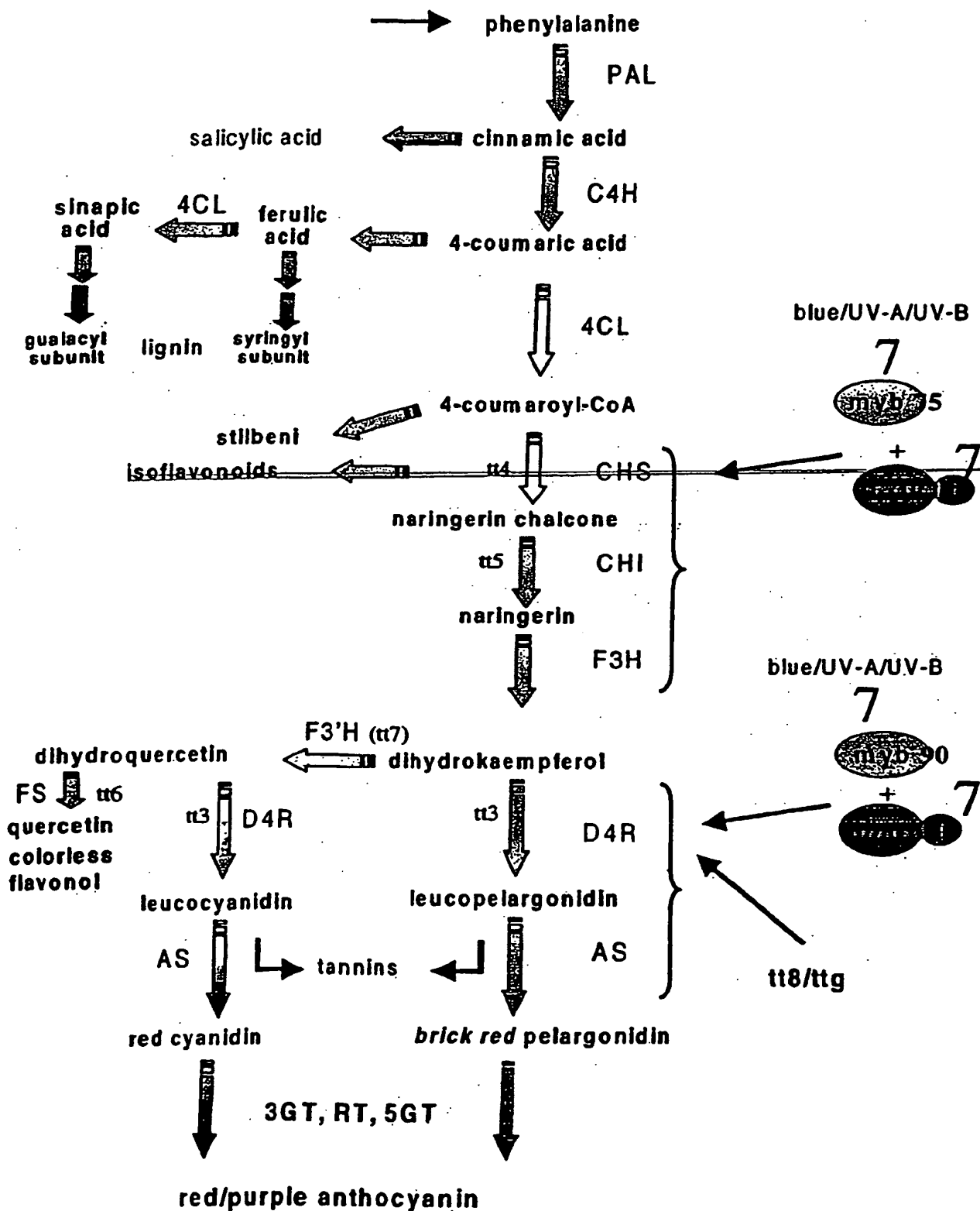
L: plants grown for 6 weeks in photoperiod 16 hours light/8 h dark

D: plants grown for 6 weeks in photoperiod 16 hours light/8 h dark and then preadapted to

FIG. 16.

phenylpropanoid metabolic pathway

FIG 16



SEQUENCE LISTING

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35     40     45
Cys Ser Lys Ser Cys Arg Leu Arg Trp Thr Asn Tyr Leu Arg Pro Gly
50     55     60
Ile Lys Arg Gly Asn Phe Thr Pro His Glu Glu Gly Met Ile Ile His
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 Ser Thr Tyr Ala Ser Ser Thr Glu Asn Ile Ser Arg Leu Val Glu Gly
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 His Lys Met Gln Asn Arg Thr Asn Asn Phe Ile Asp His His Ser Asp
 180 185 190
 Gln Phe Pro Tyr Glu Gln Leu Gln Gly Ser Arg Glu Glu Gly His Ser
 195 200 205
 Lys Gly Ile Asn Gly Asp Asp Gln Gly Ile Lys Asn Ser Glu Asn
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 Asp Asp His Asn Ala Thr Pro Pro Leu Thr Phe Ile Glu Lys Trp Leu
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 Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Ile
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 aga aaa aga ctt cta aag atg gga atc gac ccg gtt aca cac act cca 193
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 aac tct tcg cat cat cat cat cat cat cat caa caa cat atg aac atg 289
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16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/30503

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) A01H 1/00, 9/00, 11/00; C07H 21/04; C12N 5/04, 5/10, 15/00, 15/09, 15/63, 15/70, 15/74, 15/82, 15/87
US CL 435/ 320.1, 419, 468; 536/ 23.6; 800/ 278, 295

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/ 320.1, 419, 468; 536/ 23.6; 800/ 278, 295

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	KRANZ et al. Towards functional characterisation of the members of the R2R3-MYB gene family from Arabidopsis thaliana. The Plant Journal. 1998, Vol. 16, No. 2, pages 263-276, especially pages 264-267 Table 1, page 271 column 2 2nd full paragraph, pages 272-273 Table 2.	1-22, 109-114 ----- 38-86, 95-100, 103-108, 115, 119-120
X --- Y	GenBank, Accession AF062895, KRANZ et al. Towards functional characterisation of the members of the R2R3-MYB gene family from Arabidopsis thaliana. The Plant Journal. 1998, Vol. 16, No. 2, pages 263-276, especially page 266 Table 1 (X and Y).	1-22, 109-114 ----- 38-86, 95-100, 103-108, 115, 119-120
X --- Y	GenBank, Accession AF062907, KRANZ et al. Towards functional characterisation of the members of the R2R3-MYB gene family from Arabidopsis thaliana. The Plant Journal. 1998, Vol. 16, No. 2, pages 263-276, especially page 266 Table 1 (X and Y).	1-22, 109-114 ----- 38-86, 95-100, 103-108, 115, 119-120
X --- Y	GenBank, Accession AF062908, KRANZ et al. Towards functional characterisation of the members of the R2R3-MYB gene family from Arabidopsis thaliana. The Plant Journal. 1998, Vol. 16, No. 2, pages 263-276, especially page 266 Table 1 (X and Y).	1-22, 109-114 ----- 38-86, 95-100, 103-108, 115, 119-120

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

16 January 2001 (16.01.2001)

Date of mailing of the international search report

07 MAR 2001

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703)305-3230

Authorized officer

Cynthia Collins

Telephone No. (703) 308-01

TERRY J. DEY
PARALEGAL SPECIALIST
TECHNOLOGY CENTER 1600

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/30503

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---	GenBank. Accession AF062915. KRANZ et al. Towards functional characterisation of the members of the R2R3-MYB gene family from <i>Arabidopsis thaliana</i> . <i>The Plant Journal</i> . 1998, Vol. 16, No. 2, pages 263-276, especially page 267 Table 1 (X and Y).	1-22, 109-114
Y		38-86, 95-100, 103-108, 115, 119-120
P.Y	SHIMIZU et al. Molecular cloning and characterization of a subfamily of UV-B responsive MYB genes from soybean. <i>Breeding Science</i> . June 2000, Vol. 50, pages 81-90, especially page 83 Figure 1, page 86 column 2 first and second full paragraphs, page 87 Figure 3, page 88 Figure 5, page 89 Figure 6.	1-22, 38-86, 95-100, 103-115, 119-120
Y	SHINOZAKI et al. Molecular responses to water stress in <i>Arabidopsis thaliana</i> . <i>J. Plant Res.</i> June 1998, Vol. 111, pages 345-351, especially page 347 column 2 first full paragraph, page 348 Figure 4.	38-86, 95-100, 103-108, 115, 119-120
X ---	KIRIK et al. Ectopic expression of a novel MYB gene modifies the architecture of the <i>Arabidopsis</i> inflorescence. <i>The Plant Journal</i> . March 1998, Vol. 13, No. 6, pages 729-742, especially page 731 Figure 1, page 734 Figure 5, page 736 Figure 7, page 737 paragraph spanning columns 1 and 2, page 740 Vector constructs and plant transformation.	95-100, 103-108
Y		1-22, 38-86, 109-115, 119-120
Y	ABE et al. Role of <i>Arabidopsis</i> MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. <i>The Plant Cell</i> . October 1997, Vol. 9, pages 1859-1868, especially page 1863 Figure 4A, page 1865 Figure 6 and column 2 second full paragraph, page 1866 Figure 7.	38-86, 95-100, 103-115, 119-120
Y	LOIDL et al. Oncogene- and tumor-suppressor gene-related proteins in plants and fungi. <i>Critical Reviews in Oncogenesis</i> . 1996, Vol. 7, Nos: 1 and 2, pages 49-64, especially pages 51-52 A. Myb-Related Genes/Proteins, page 52 Table 1.	38-86, 95-100, 103-108, 115, 119-120
Y	ITURRIAGA et al. A family of novel myb-related genes from the resurrection plant <i>Craterostigma plantagineum</i> are specifically expressed in callus and roots in response to ABA or dessication. <i>Plant Molecular Biology</i> . November 1996, Vol. 32, pages 707-716, especially pages 711 Figure 3, page 713 Figure 6, page 114.	1-22, 38-86, 95-100, 103-115, 119-120
Y	SCHAEFFER et al. Identification of enhancer and silencer regions involved in salt-responsive expression of Crassulacean acid metabolism (CAM) genes in the facultative halophyte <i>Mesembryanthemum crystallinum</i> . <i>Plant Molecular Biology</i> . May 1995, Vol. 28, pages 205-218, especially page 209 Figure 1, page 213 Figure 4, page 215 column 2 first full paragraph, page 216 column 1 paragraph spanning pages 215-216 and paragraph spanning columns 1 and 2.	38-86, 95-100, 103-108, 115, 119-120
Y	YAMAGUCHI-SHINOZAKI et al. Regulation of genes that are induced by drought stress in <i>Arabidopsis thaliana</i> . <i>J. Plant Research</i> . 1995, Vol. 108, pages 127-136, especially page 128 Figure 1, page 133 column 2 first paragraph - page 135 column 1 first paragraph.	1-22, 38-86, 95-100, 103-115, 119-120
Y	YAMAGUCHI-SHINOZAKI et al. Function and regulation of genes that are induced by dehydration stress in <i>Arabidopsis thaliana</i> . <i>JIRCAS Journal</i> . 1994, Vol. 1, pages 69-79, entire article.	1-22, 38-86, 95-100, 103-115, 119-120
Y	URAO et al. An <i>Arabidopsis</i> myb homolog is induced by dehydration stress and its gene product binds to the conserved MYB recognition sequence. <i>The Plant Cell</i> . November 1993, Vol. 5, pages 1529-1539, especially page 1530 Figure 1, page 1531 Figure 2, page 1532 Figures 3 and 4.	1-22, 38-86, 95-100, 103-115, 119-120

Form PCT/ISA/210 (continuation of second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/30503

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-22, 38-86, 95-100, 103-115, 119-120

Remark on Protest

☐
☐

- The additional search fees were accompanied by the applicant's protest.
No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/30503

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-22, 38-86, 95-100, 103-115, 119, and 120, drawn to compounds comprising a MYB nucleic acid molecule.

Group II, claim(s) 23-27, drawn to MYB polypeptides.

Group III, claim(s) 28-32, drawn to antibodies against MYB polypeptides.

Group IV, claim(s) 33-37, drawn to variant MYB polypeptides.

Group V, claim(s) 87-94, drawn to a method of producing a stress sensitive transgenic plant.

Group VI, claim(s) 101-102, drawn to a method of screening a plant for stress tolerance.

Group VII, claim(s) 117-118, drawn to a method of assaying environmental conditions of a field.

Group VIII, claim(s) 116, drawn to a method of inhibiting the expression of MYB genes in a plant cell.

The inventions listed as Groups I-VIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Groups I-VIII lack the same special technical feature in that the products differ structurally and functionally from one another, and the methods result in different products or uses. The products of Group I are compounds comprising a MYB nucleic acid molecule, which is not a special technical feature of the products of Groups II-IV. The products of Group II are MYB polypeptides, which is not a special technical feature of the products of Groups I, III, and IV. The products of Group III are antibodies against MYB polypeptides, which is not a special technical feature of the products of Groups I, II, and IV. The products of Group IV are variant MYB polypeptides, which is not a special technical feature of the products of Groups I-III. The method of Group V is used to make stress sensitive plants, which is not a special technical feature of the methods of Groups VI-VIII. The method of Group VI is used to identify stress tolerant plants, which is not a special technical feature of the methods of Groups V, VII, and VIII. The method of Group VII is used to identify the environmental conditions of a field, which is not a special technical feature of the methods of Groups V, VI, and VIII. The method of Group VIII is used to inhibit MYB gene expression, which is not a special technical feature of the methods of Groups V-VII. Therefore, lack of unity between the stated groups is properly made.

Continuation of B. FIELDS SEARCHED Item 3: WEST & STN(AGRICOLA, BIOSIS, BIOTECHNO, BIOTECHDS, BIOTECHABS, CABA, CAPLUS, EMBASE, MEDLINE, SCISEARCH) search terms: plant transcription factor, myb, Arabidopsis, stress, inventor name ; STIC SEQUENCE SEARCH SEQ ID NOS:1-8.

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
10 May 2001 (10.05.2001)

PCT

(10) International Publication Number
WO 01/32002 A1

(51) International Patent Classification⁷: A01H 1/00, 9/00,
11/00, C07H 21/04, C12N 5/04, 5/10, 15/00, 15/09, 15/63,
15/70, 15/74, 15/82, 15/87

(21) International Application Number: PCT/US00/30503

(22) International Filing Date:
6 November 2000 (06.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/163,579 5 November 1999 (05.11.1999) US
09/693,855 23 October 2000 (23.10.2000) US

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(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE,
DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU,
ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO,
NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR,
TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report

(48) Date of publication of this corrected version:
16-May 2002

(15) Information about Correction:
see PCT Gazette No. 20/2002 of 16 May 2002; Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/32002 A1

(54) Title: MYB TRANSCRIPTION FACTORS AND USES THEREOF

(57) Abstract: Nucleic acids that encode stress tolerance-related MYB polypeptides in plants are described. More particularly, the present invention relates to nucleotides that encode MYB transcription factors, preferably the following MYB transcription factors: MYB60, MYB74, MYB90. The present invention also relates to the MYB polypeptides themselves, as well as to variants and antibodies thereof. The invention further relates to uses of MYB transcription factors and to plants transformed by the nucleic acids. Additionally, the present invention relates to the production of stress-sensitive plants, which may be preferably used as environmental monitors.

MYB Transcription Factors and Uses Thereof

Field of the Invention

The present invention relates to nucleic acids, which encode stress tolerance-related or stress sensitivity-related myloblastosis (MYB) polypeptides in plants.

- 5 More particularly, the present invention relates to nucleic acids that encode MYB transcription factors or antisense molecules complementary to MYB transcription factors, preferably the following MYB transcription factors: MYB60, MYB74, MYB75, and MYB90. The present invention also relates to the MYB polypeptides themselves, as well as to variants and antibodies thereof. The invention further
- 10 relates to uses of MYB transcription factors and to plants transformed by the nucleic acids. The invention also relates to transgenic plants containing the MYB nucleic acids in antisense orientation.

Background of the Invention

- 15 Plant stresses such as drought, high salt concentration and high and low temperature are some of the most important factors affecting plant distribution on the earth surface. Identification of genes involved in mechanisms through which plants adapt to adverse conditions is an important goal for future improvement of crop species in their tolerance to stress, such as dehydration. Some genes involved
- 20 in water stress response present myloblastosis (MYB) recognition sites in their promoter regions. MYB proteins are a class of transcription factors, identified in nearly all eukaryotes, sharing a common DNA binding domain.

The so-called MYB domain includes two or three imperfect repeats of 50-53 amino acids (R1, R2 and R3) and is well conserved between MYB proteins of animals, yeast and plants. Although there are plant MYB-like proteins containing only one repeat, the DNA binding domain encoded by most of the plant MYB genes is formed by two repeats, which are most similar to repeats R2 and R3 of the animal cMYB proteins. Thus, MYB-related proteins from plants generally contain two related helix-turn-helix motifs, the R2 and R3 repeats. It has been suggested that MYB genes play an important role in the regulation of secondary metabolism, the control of cell shape, disease resistance, and hormone responses.

Land plants are exposed to many types of abiotic stress. One of these is dehydration, which can derive from drought, low temperature and high salt concentration in the soil. Because under those adverse environmental conditions plant growth and survival are seriously affected, series of mechanisms evolved to respond and adapt to osmotic stress. Under water-stress conditions plant cells lose water and decrease turgor pressure. The plant hormone abscisic acid (ABA) increases as a result of water stress. ABA plays an important role in the tolerance of plants to drought, high salinity and cold. Water deficit is a normal component of some developmental processes in plants, such as seed development, common to most higher plants. Such a water deficit results in changes in cell volume and membrane shape, disruption of water potential gradients and membrane integrity, protein denaturation and changes in osmolyte concentration.

The ability of plants to survive cellular water deficit depends on the species and genotype, the length and severity of water loss, the age and stage of development and the organ and cell type. Responses to water deficit may occur within seconds, such as modifications in membrane potential and in the phosphorylation status of proteins, or within minutes and hours, such as changes in protein composition and gene expression.

The first functionally characterized MYB proteins in plants, *C1* and *Pl*, control phenylpropanoid biosynthesis in maize. Others play a role in the regulation of cell shape or in trichomes and root hair differentiation. MYB genes are involved in

the plant response to chemical messengers such as salicylic acid and hormones or in the response to different external challenges and stimuli, such as light and biotic or abiotic stresses. In general, this family participates in the control of a widespread range of functions, related to plant growth, development and interactions with the environment.

It has been estimated that the plant *Arabidopsis thaliana* contains more than 100 R2R3-MYB genes. Information obtained from studying *Arabidopsis* can be applied to other flowering plants, such as those grown for fiber or food. For instance, once a gene has been discovered in *Arabidopsis*, the equivalent gene may be found more easily in other plants. Thus, the function of many genes isolated from crop plants can be better understood by studying their *Arabidopsis* homologues. Thus, knowledge of *Arabidopsis* has led to a better understanding of all higher plants, and to the development of disease-resistant plants in other species.

The characterization of transcription factors that control the coordinate expression of multiple genes involved in stress response is very important with respect to improving plant tolerance.

Summary of the Invention

This invention is based on the cloning of full length cDNA clones encoding MYB transcription factors that result in enhanced stress tolerance in plants. The present invention also relates to the role of certain MYB genes in the control of the flavonoid and phenylpropanoid pathways. The nucleotide sequences, antisense sequences and corresponding amino acid sequences are disclosed herein.

The present invention relates to nucleic acid molecules that encode MYB transcription factors, complementary antisense nucleic acids, the MYB transcription factors themselves, and variants and antibodies thereof. Preferred MYB transcription factors according to the present invention are MYB60, MYB74, MYB75, and MYB90. Certain MYB transcription factors are included in a journal article, "Towards functional characterisation of the members of the R2R3-MYB gene family from *Arabidopsis thaliana*," *The Plant Journal*, 16(2), 263-276 (1998),

which is herein incorporated by reference in its entirety. The present invention also relates to uses of MYB transcription factors. Preferred uses include producing stress tolerant plants and in the case of antisense, producing stress sensitive plants that may preferably act as environmental monitors. The present invention further relates to
5 plants transformed by vectors from such nucleic acid molecules.

The present invention provides a method for genetic modification of plants to control the stress tolerance of plants, for example to drought, temperature and salt, or to increase the stress sensitivity of plants, such that they may be used as environmental monitors.

10 In one aspect, the present invention is directed to nucleic acid molecules that comprise a sequence encoding a stress tolerance-related MYB transcription factor in a plant. Preferably, the MYB transcription factor is selected from the group of MYB60, MYB74, MYB75 and MYB90. Even more preferably, the nucleic acid has a sequence that encodes one of SEQ ID NOs. 2, 4, 6 or 8.

15 In another embodiment of the invention, the present invention is directed to an isolated nucleic acid molecule that has sequence that encodes a plant stress tolerance-related MYB transcription factor. Preferably the MYB transcription factor is one of the following transcription factors: MYB60, MYB74, MYB75 and MYB90. Even more preferably, the DNA molecule hybridizes under low stringency
20 conditions with one of the following nucleic acid sequences SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7 or a variant of the isolated nucleic acid molecule.

In another embodiment of the invention, there is provided a MYB polypeptide that is a plant stress tolerance-related MYB transcription factor. A
25 preferred MYB polypeptide has the amino acid sequence of one of SEQ ID NOs: 2, 4, 6 or 8, or is a variant thereof. Also encompassed by the present invention are variants and antibodies of the polypeptides of the present invention.

The invention is further directed to a vector for transformation of plant cells.

The invention also provides a plant cell transformed with the vector as
30 described above, a plantlet, mature plant or seeds generated from such a cell, or a

plant part of such a plantlet or plant. Also provided is a method of producing a plant having enhanced stress tolerance, or in the case of antisense, producing a plant having increased sensitivity, by transforming the plant. Plants and seeds produced as described herein, or progeny, hybrids, clones or plant parts preferably exhibit

5 increased stress tolerance or increased stress sensitivity.

Further provided are methods for enhancing a plant's tolerance to stress, or in the case of antisense, increasing stress sensitivity, by transforming the plant with a vector described herein.

The nucleic acids, polypeptides, variants, antibodies, seeds and plants of the

10 present invention may also be useful as research tools. They should find broad applications in the generation of transgenic plants with enhanced tolerance to stress and enhanced sensitivity to stress.

Brief Description of the Drawings

15 Figure 1 shows a cDNA sequence encoding a AtMYB60 polypeptide (SEQ ID NO:1), as well as the corresponding amino acid sequence (SEQ ID NO:2).

Figure 2 shows a cDNA sequence encoding a AtMYB74 polypeptide (SEQ ID NO:3), as well as the corresponding amino acid sequence (SEQ ID NO:4).

Figure 3A shows a cDNA sequence encoding a AtMYB75 polypeptide (SEQ

20 ID NO:5).

Figure 3B shows an amino acid sequence (SEQ ID NO:6) of an AtMYB75 polypeptide.

Figure 4 shows a cDNA sequence encoding a AtMYB90 polypeptide (SEQ ID NO:7), as well as a corresponding amino acid sequence (SEQ ID NO:8).

25 Figure 5 shows an RT-PCR analysis of AtP5CS1.

Figure 6 shows an RT-PCR analysis of RD22.

Figure 7 shows an RT-PCR analysis of erd10.

Figure 8 shows an RT-PCR analysis of ADH1.

Figure 9 shows an RT-PCR analysis of AtMYB74.

30 Figure 10 shows an RT-PCR analysis of AtMYB75.

Figure 11 shows the expression patterns of AtMYB75, AtMYB74, ERD10, ADH1, P5CS1, and RD22, following treatment with PEG 30% and COLD 4°C.

Figure 12 shows an RT-PCR analysis of AtMYB90.

Figure 13 shows an RT-PCR analysis of AtMYB60.

5 Figure 14 shows the expression patterns of AtMYB74, ADH1, P5CS1, ERD10, RD22e, and AtMYB60, following treatment with ABA 100µM.

Figure 15 shows a comparison between the expression of MYB75 and MYB90 genes and structural genes of the phenylpropanoid pathway after light treatments. Specifically, Figure 15 shows MYB75 and MYB90 expression patterns
10 in response to white, blue, UV-A and UV-B light.

Figure 16 shows a metabolic pathway of phenylpropanoid and how MYB75 and MYB90 are believed to be involved in the pathway.

Detailed Description

15 Identification of genes involved in mechanisms through which plants adapt... to adverse conditions such as drought causing conditions, may improve crop species in their tolerance to stress, such as dehydration and high salt conditions, and may thus, increase the yield of a crop. Some genes involved in water stress response present MYB recognition sites in their promoter regions. MYB proteins are a class
20 of transcription factors, identified in nearly all eukaryotes, sharing a common DNA binding domain that is highly conserved in all eukaryotes. The binding domain consists of different repeats of a helix-turn-helix motif. In animals these factors represent a small gene family involved in the control of cell proliferation and in the prevention of apoptosis. In plants these proteins form the biggest regulatory family
25 so far known, with more than 100 members identified in *Arabidopsis thaliana*, whose functions remain mainly unknown.

Applicants have identified certain MYB genes, including MYB60, MYB74, MYB75 and MYB90, which are particularly useful with regard to manipulation of stress tolerance and stress sensitivity in plants. The expression of certain genes

(*erd10*, *rd22*, *ADHI* and *AtP5CS1*) known to be involved in osmotic stress response are also described herein.

Full length cDNA sequences encoding MYB transcription factors relating to stress tolerance have been isolated by reverse transcriptase mediated polymerase chain reaction (RT-PCR). These sequences are provided herein.

Additionally, Applicants believe that the MYB75 and MYB90 genes are involved in the control of the flavonoid and anthocyanin pathways, that MYB74 is a transcription factor that is activated during stress, and the MYB60 is a transcription factor that is repressed during stress.

10

Definitions

As used herein, the term "plant" refers to either a whole plant, a plant part, a plant cell or a group of plant cells or progeny of any thereof. This term includes, but is not limited to, whole plants, plant parts, plant cells, plant organs, plant seeds, plant progeny, propagules, protoplasts, callus, cell cultures and any groups of plant cells organized into structural and/or functional units. The type of plant which can be used in the methods of the invention is not limited and includes, for example, ethylene-sensitive and ethylene-insensitive plants; fruit bearing plants such as apricots, apples, oranges, bananas, grapefruit, pears, tomatoes, strawberries, avocados, etc.; vegetables such as carrots, peas, lettuce, cabbage, turnips, potatoes, broccoli, asparagus, etc.; flowers such as carnations, roses, mums, etc.; agronomic crops such as corn, rice, soybean, alfalfa and the like; and in general, any plant that can take up and express the DNA molecules of the present invention. It may include plants of a variety of ploidy levels, including haploid, diploid, tetraploid and polyploid. The plant may be either a monocot or dicot.

The term "plant" also includes tissue of a plant in planta or in culture. Plant parts include, but are not limited to, leaves, stems, roots, and flowers. Plant cell progeny should be understood as referring to any cell or tissue derived from plant cells including callus; plants; plant seed; pollen; plant embryos; and plant parts such as stems, roots, fruits, leaves or flowers. Propagules should be understood as

referring to any plant tissue capable of being sexually or asexually propagated, or being propagated *in vivo* or *in vitro*. Such propagules preferably consist of the protoplasts, cells, calli, tissues, embryos or seeds of the regenerated plants. The use of the term "plant" in conjunction with, or in the absence of, any specific type of plant as listed above or otherwise embraced by this definition is not intended to be exclusive of any other type of plant, plant part or progeny thereof.

The term "transgenic" refers to organisms (plants or animals) into which new DNA sequences are integrated. A "transgenic plant" is defined herein as a plant which is genetically modified in some way, including but not limited to a plant which has incorporated heterologous or homologous stress tolerance-related nucleic acid molecule, such as DNA or modified DNA, into its genome. The altered genetic material may encode a protein or antisense molecule, for example. A "transgene" or "transgenic sequence" is defined as a foreign gene or partial sequence which has been incorporated into a transgenic plant.

The term "hybridization" as used herein is generally used to mean hybridization of nucleic acids at appropriate conditions of stringency as would be readily evident to those skilled in the art depending upon the nature of the probe sequence and target sequences. Conditions of hybridization and washing are well known in the art, and the adjustment of conditions depending upon the desired stringency by varying incubation time, temperature and/or ionic strength of the solution are readily accomplished. See, for example, Sambrook, J. et al., *Molecular Cloning: A Laboratory Manual*, 2nd edition, Cold Spring Harbor Press, Cold Spring Harbor, New York, 1989. The choice of conditions is partly dictated by the length of the sequences being hybridized, in particular, the length of the probe sequence, the relative G-C content of the nucleic acids and the amount of mismatches to be permitted. Low stringency conditions are preferred when partial hybridization between strands that have lesser degrees of complementarity is desired. When perfect or near perfect complementarity is desired, high stringency conditions are preferred. For typical high stringency conditions, the hybridization solution contains 6X S.S.C., 0.01 M EDTA, 1X Denhardt's solution and 0.5% SDS. Hybridization is

carried out at about 68°C for about 3 to 4 hours for fragments of cloned DNA and for about 12 to about 16 hours for total eukaryotic DNA. For lower stringencies the temperature of hybridization is reduced to about 42°C below the melting temperature (T_M) of the duplex. The T_M is known to be a function of the G-C
5 content and duplex length as well as the ionic strength of the solution.

"High stringency conditions" should be understood to be those conditions normally used by one of skill in the art to establish at least about a 90% sequence identity between complementary pieces of DNA or DNA and RNA. Lesser sequence identity, such as at least about 50% sequence identity or preferably at least about
10 70% may also be desired and obtained by varying the hybridization conditions such that the conditions are "low stringency conditions".

As used herein, the term "substantial sequence identity" or "substantial homology" is used to indicate that a nucleotide sequence or an amino acid sequence exhibits substantial structural or functional equivalence with another nucleotide or
15 amino acid sequence. Any structural or functional differences between sequences having substantial sequence identity or substantial homology will be *de minimis*; that is, they will not affect the ability of the sequence to function as indicated in the desired application. Differences may be due to inherent variations in codon usage among different species, for example. Structural differences are considered *de*
20 *minimis* if there is a significant amount of sequence overlap or similarity between two or more different sequences or if the different sequences exhibit similar physical characteristics even if the sequences differ in length or structure. Such characteristics include, for example, ability to hybridize under defined conditions, or in the case of proteins, immunological crossreactivity, similar enzymatic activity,
25 etc. For example, DNA or amino acid sequences having substantial sequence identity may share about 50% to about 100% sequence identity, preferably about 65% to about 99% sequence identity, and most preferably about 70% to about 99% sequence identity. Sequence identity determinations can be performed for example, using the FASTA program (Genetics Computer Group Madison, Wis.).
30 Alternatively, identity similarity determinations can be performed using BLASTP

(Basic Local Alignment Search Tool) of the Experimental GENINFO Blast Network Service. See also Pasternak, et al. Methods in Plant Molecular Biology and Biotechnology, Glick, et al. (eds.), pages 251-267 (CRC Press, 1993). Sequence identity also includes a relationship wherein one or several subsequences of
5 nucleotides or amino acids are missing, or subsequences with additional nucleotides or amino acids are interdispersed.

The minimal amount of sequence identity required by the present invention is that sufficient to result in sufficient complementarity to provide recognition of the specific target RNA or DNA and in the case of antisense molecules inhibition or
10 reduction of its transcription, translation or function while not affecting function of other RNA or DNA molecules and the expression of other genes.

Additionally, two nucleotide sequences are "substantially complementary" if the sequences have at least about 50 percent, more preferably, at least about 70 percent and most preferably at least about 90 percent sequence similarity between
15 them. Two amino acid sequences have a substantial sequence identity if they have at least about 50%, preferably about 70% or more similarity between the active portions of the polypeptides.

The term "functional derivative" of a nucleic acid (or poly- or oligonucleotide) is used herein to mean a fragment, variant, homolog, or analog of
20 the gene or nucleotide sequence encoding a stress tolerance-related MYB transcription factor. A functional derivative may retain at least a portion of the function of the stress tolerance-related encoding DNA which permits its utility in accordance with the invention.

A "fragment" of the gene or DNA sequence refers to any subset of the
25 molecule, e.g., a shorter polynucleotide or oligonucleotide of an amino acid or nucleotide sequence that retains some desired chemical or biological property of the full-length sequence such that use of the full-length sequence is not necessary to achieve the desired purpose. A "variant" refers to a molecule substantially similar to either the entire gene or a fragment thereof, such as a nucleotide substitution variant
30 having one or more substituted nucleotides, but which maintains the ability to

hybridize with the particular gene or to encode mRNA transcript which hybridizes with the native DNA.

A "homolog" refers to a fragment or variant sequence from a different plant genus or species. An "analog" refers to a non-natural molecule substantially similar to or functioning in relation to either the entire molecule, a variant or a fragment thereof.

The term "operably linked" refers to components of a chimeric gene or an expression cassette that function as a unit to express a heterologous protein. For example, a promoter operably linked to a heterologous DNA, which encodes a protein, promotes the production of functional mRNA corresponding to the heterologous DNA.

"Functional derivatives" of the stress tolerance-related MYB polypeptides as described herein are fragments, variants, analogs, or chemical derivatives of stress tolerance-related MYB polypeptides, which retain at least a portion of the stress tolerance-related or immunological cross reactivity with an antibody specific for MYB. A fragment of the stress tolerance-related MYB polypeptide refers to any subset of the molecule. Variant peptides may be made by direct chemical synthesis, for example, using methods well known in the art. An analog of stress tolerance-related polypeptide refers to a non-natural protein substantially similar to either the entire protein or a fragment thereof. Chemical derivatives of a stress tolerance-related MYB polypeptide contain additional chemical moieties not normally a part of the peptide or peptide fragment. Modifications may be introduced into the stress tolerance-related MYB peptide or fragment thereof, for example, by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues.

A "chimeric" sequence or gene is a DNA sequence containing at least two heterologous parts, e.g., parts derived from naturally occurring DNA sequences which are not associated in their naturally occurring states, or containing at least one part that is of synthetic origin and not found in nature.

With reference to nucleic acids of the invention, the term "isolated nucleic acid" is sometimes used. This term, when applied to DNA, refers to a DNA molecule that is substantially separated from other nucleic acid sequences found in the cell. For example, the "isolated nucleic acid" may comprise a DNA molecule
5 inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a procaryote or eucaryote. Recombinant plasmids or vectors containing novel MYB genes that may be propagated in for example, *E. coli*, *S. cerevisiae* and *Agrobacteria* are contemplated for use in the present invention. These vectors may optionally contain strong constitutive promoter elements to facilitate
10 high expression of the MYB genes of the invention. Alternatively, they may contain inducible promoter elements so that expression of the MYB genes of the invention can be controlled by addition of an inducer compound.

With respect to RNA molecules of the invention, the term "isolated nucleic acid" primarily refers to an RNA molecule encoded by an isolated DNA molecule as
15 defined above. Alternatively, the term may refer to an RNA molecule that has been sufficiently separated from RNA molecules with which it would be associated in its natural state (i.e., in cells or tissues), such that it exists in a "substantially pure" form.

A "substantially pure" nucleic acid sequence is defined herein as a DNA or
20 RNA molecule (sequence) isolated in substantially pure form from a natural or non-natural source. Such a molecule may occur in a natural system, for example, in bacteria, viruses or in plant or animal cells, or may be provided, for example, by synthetic means or as a cDNA. Substantially pure DNA or RNA sequences are typically isolated in the context of a cloning vector. "Substantially pure" means that
25 DNA or RNA molecules other than the ones intended are present only in marginal amounts, for example less than 5%, less than 1%, or preferably less than 0.1%. Substantially pure DNA or RNA sequences and vectors containing may be, and typically are, provided in solution, for example in aqueous solution containing buffers or in the usual culture media.

Nucleic acid molecules of the present invention may be single stranded or double stranded or may be a DNA or RNA, or hybrids thereof.

Nucleic Acid Molecules

5 The present invention relates to a compound comprising a nucleic acid molecule that encodes a MYB transcription factor or is complementary to at least a portion of a MYB gene. The MYB transcription factor may be a stress tolerance-related MYB polypeptide. Preferred MYB transcription factors that are encoded by the nucleic acid molecule of the present invention are MYB60, MYB74, MYB75,
10 and MYB90 polypeptides. Preferably the nucleic acid is DNA that encodes an amino acid sequence having SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. A preferred embodiment of the present invention includes nucleic acid molecules that encode a MYB transcription factor, which shares about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ
15 ID NO:8. More preferably, the nucleic acid molecules encode a MYB transcription factor, which shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the nucleic acid molecules encode a MYB transcription factor, which shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ
20 ID NO:8.

Also encompassed by the present invention are isolated nucleic acid molecules having a sequence that encodes a plant stress tolerance-related MYB transcription factor. Preferably the MYB transcription factor is one of the following transcription factors: MYB60, MYB74, MYB75 and MYB90. Even more
25 preferably, the DNA molecule hybridizes under low stringency conditions with one of the following nucleotide sequences: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7 or a functional derivative or variant of the isolated nucleic acid molecule.

In a preferred embodiment of the invention, isolated nucleic acid molecules
30 encompassed by the present invention are those that encode a MYB transcription

factor, which shares about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. More preferably, the DNA molecules encode a MYB transcription factor, which shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the DNA molecules encode a MYB transcription factor, which shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

An aspect of the present invention disclosed herein provides for novel cDNA clones coding for MYB polypeptides. These cDNAs, or their genomic counterparts, or DNA molecules with substantial sequence identity to either, can be engineered for expression of the encoded MYB polypeptides and transformed into plants that have enhanced stress tolerance or, in the case of antisense, plants that are stress sensitive, as described herein.

MYB encoding nucleic acid molecules of the invention include cDNA, genomic DNA, RNA, and fragments thereof which may be single- or double-stranded. Thus, this invention provides oligonucleotides having sequences capable of hybridizing with at least one sequence of a nucleic acid molecule of the present invention, such as selected segments of the cDNA having SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7. Such oligonucleotides are useful as probes for detecting or isolating MYB genes in other plant species.

Also provided herein are compounds comprising antisense nucleic acid molecules encoding an RNA molecule which is complementary to at least a portion of an RNA transcript of the DNA molecule described herein above, wherein the encoded RNA molecule hybridizes with the RNA transcript such that expression the MYB transcription factor is altered. The antisense nucleic acid molecule can be full length or only a portion of the nucleic acid sequence.

The antisense nucleic acid molecule is substantially homologous to at least a portion of a DNA molecule encoding a MYB transcription factor. In a preferred embodiment, the DNA molecule encoding a MYB transcription factor hybridizes with SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7, or is

substantially homologous to at least a portion of an RNA sequence encoded by the DNA molecule encoding a MYB transcription factor. In one embodiment of the invention, the antisense nucleic acid molecule is substantially homologous to at least a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7, or the
5 RNA transcript encoded by SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7. In another embodiment, the antisense nucleic acid molecule is substantially homologous to at least a portion of the 5' non-coding portion of a DNA molecule encoding a MYB transcription factor, wherein the DNA molecule hybridizes with SEQ ID NO:1 SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7.

10 Antisense oligonucleotides are preferably at least about six nucleotides in length to provide minimal specificity of hybridization and may be complementary to DNA or mRNA encoding a MYB transcription factor or a portion thereof. The antisense oligonucleotide may extend in length up to and beyond the full coding sequence for which it is antisense. The antisense oligonucleotides can be DNA or
15 RNA or chimeric mixtures or derivatives or modified versions thereof, single stranded or double stranded.

The action of the antisense oligonucleotide may result in alteration, primarily inhibition, of MYB expression in cells. For a general discussion of antisense see: Alberts, et al., Molecular Biology of the Cell, 2nd ed., Garland Publishing, Inc. New
20 York, New York, 1989 (in particular pages 195-196, incorporated herein by reference).

The antisense oligonucleotide may be complementary to any portion of the MYB gene. In one embodiment, the antisense oligonucleotide may be between 6 and 100 nucleotides in length, and may be complementary to the 5'-non-coding
25 sequence of the senescence-induced DHS sequence, for example. Antisense oligonucleotides primarily complementary to 5'-non-coding sequences are known to be effective inhibitors of expression of genes encoding transcription factors. Branch, M.A., Molec. Cell Biol., 13:4284-4290 (1993).

Preferred antisense nucleotides are substantially homologous to a portion of
30 the mRNA encoding MYB transcription factors. For example, introduction of the

full length cDNA clone encoding MYB transcription factors in an antisense orientation into a plant is expected to result in successful altered MYB gene expression. Moreover, introduction of partial sequences, targeted to specific portions of the MYB gene, can be equally effective.

5 The minimal amount of homology required by the present invention is that sufficient to result in sufficient complementarity to provide recognition of the specific target RNA or DNA and inhibition or reduction of its translation or function while not affecting function of other RNA or DNA molecules and the expression of other genes. While the antisense oligonucleotides of the invention comprise
10 sequences complementary to at least a portion of an RNA transcript of the MYB gene, absolute complementarity, although preferred is not required. The ability to hybridize may depend on the length of the antisense oligonucleotide and the degree of complementarity. Generally, the longer the hybridizing nucleic acid, the more base mismatches with the MYB target sequence it may contain and still form a
15 stable duplex. One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting temperature of the hybridized complex, for example.

Also encompassed by the present invention are nucleic acid molecules (sense and antisense) that may be modified at the sugar, base or phosphate. Those in the art
20 will recognize that one or more bases in a nucleotide sequence may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Modified bases may include for example, synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and
25 other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-
30 trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and

7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

Recombinant Vectors

5 The present invention is further directed to a recombinant vector from any of the nucleic acid molecules encoding the MYB transcription factors described above and to a recombinant vector from any of the antisense nucleic acid molecules described above.

 Vectors are recombinant DNA sequences which may be used for isolation
10 and multiplication purposes of the mentioned DNA sequence and for the transformation of suitable hosts with these sequences. A vector may be a plasmid, cosmid, bacteriophage, virus or any other replicating nucleic acid that has the capability of replicating autonomously in a host cell. Preferred vectors for isolation and multiplication are plasmids which can be propagated in a suitable host
15 microorganism, for example in *E. coli*. Many vectors have been described in the art which are suitable for use as starting materials in the present invention.

 The insertion of an appropriate sequence, which is capable of transcription, into such an intermediate vector results in a vector from a chimeric DNA sequence of the invention that can then be used to transform the desired plant. Alternatively, a
20 chimeric DNA sequence can be prepared and inserted into a suitable vector which is then used to transform the desired plant.

 Vectors of the present invention can be constructed by recombinant DNA technology methods that are standard in the art. For example, the vector may be a plasmid containing a replication system functional in *Agrobacterium*. Plasmids that
25 are capable of replicating in *Agrobacterium* are well known in the art. See, Miki, et al., Procedures for Introducing Foreign DNA Into Plants, Methods in Plant Molecular Biology and Biotechnology,, Eds. B.R. Glick and J.E. Thompson. CRC Press (1993), PP. 67-83.

 With regard to antisense nucleic acid molecules, the recombinant vectors for
30 transformation of plant cells, include (a) an antisense nucleic acid molecule

substantially homologous to (1) at least a portion of a DNA molecule encoding a MYB transcription factor, such as MYB60, MYB74, MYB75 and MYB90, or (2) at least a portion of an RNA sequence encoded by the DNA molecule encoding such a MYB transcription factor; and (b) regulatory sequences operatively linked to the antisense nucleic acid molecule such that the nucleic acid molecule is expressed in a plant cell into which it is transformed.

A polynucleotide sequence (DNA, RNA) is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that polynucleotide sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the polynucleotide sequence to be expressed and maintaining the correct reading frame to permit expression of the polynucleotide sequence under the control of the expression control sequence and production of the desired sequence.

Polypeptides

Also encompassed by the present invention are stress tolerance-related MYB transcription factors. Preferred MYB transcription factors of the present invention are MYB60, MYB74, MYB75, and MYB90. In a most preferred embodiment of this aspect of the invention, the MYB transcription factor has an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

In another preferred embodiment of the invention, the MYB transcription factor shares about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8. More preferably, the MYB transcription factor shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the MYB transcription factor shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

Antibodies

According to another aspect of the invention, antibodies immunologically specific for the polypeptides described hereinabove are provided. Such antibodies include antibodies of plant MYB polypeptides. Preferably, the antibody is an
5 antibody of a MYB transcription factor. The antibody is more preferably an antibody of MYB60, MYB74, MYB75 or MYB90. In a most preferred embodiment of this aspect of the invention, the antibody is an antibody of a MYB transcription factor having an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

10 The present invention also provides antibodies, monoclonal or polyclonal, capable of immunospecifically binding to MYB proteins of the invention. Polyclonal antibodies directed toward plant stress tolerance-related MYB transcription factors may be prepared according to standard methods. In a preferred embodiment, monoclonal antibodies are prepared, which react immunospecifically
15 with various epitopes of MYB transcription factors. Monoclonal antibodies may be prepared according to general methods of Kohler and Milstein, following standard protocols. Polyclonal or monoclonal antibodies that immunospecifically interact with MYB transcription factors can be utilized for identifying and purifying such proteins. For example, antibodies may be utilized for affinity separation of proteins
20 with which they immunospecifically interact. Antibodies may also be used to immunoprecipitate proteins from a sample containing a mixture of proteins and other biological molecules.

With respect to antibodies of the invention, the term "immunologically specific" refers to antibodies that recognize and bind to one or more epitopes of a
25 polypeptide of interest (for example, MYB60), but which do not immunospecifically recognize and bind other molecules in a sample containing a mixed population of antigenic biological molecules.

30

Variants

Also encompassed by the scope of the present invention are variants of plant MYB transcription factors. Preferably, the variants of the MYB polypeptides are variants of MYB60, MYB74, MYB75, and MYB90.

- 5 Variant nucleic acid and amino acid sequences of the present invention preferably are at least about 80% identical, most preferably at least about 99% identical, to a native sequence such as the native nucleic acid sequences of SEQ ID NOs:1, 3, 5 and 7, and the native amino acid sequences of SEQ ID Nos: 2, 4, 6 and 8. Most preferred are substantially pure DNA sequences as shown in SEQ ID
- 10 NOs:1, 3, 5 and 7, and substantially pure DNA sequences having substantial sequence identity to the sequences shown in SEQ ID NOs:1, 3, 5 and 7 (see Figures 1-4). Most preferred amino acid sequences are substantially pure amino acid sequences as shown in SEQ IDNOs:2, 4, 6 and 8 and DNA sequences having substantial sequence identity to the sequences shown in SEQ ID NOs: 1, 3, 5 and 7.
- 15 For fragments, the percent identity is calculated for that portion of a native sequence that is present in the fragment.

- Variants of MYB transcription factors may also include those that share about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. More preferably, the MYB transcription factor
- 20 shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the MYB transcription factor shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

- Variants may comprise conservatively substituted sequences, that is a given
- 25 amino acid residue may be replaced by a residue having similar physiochemical characteristics. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example,

substitutions of entire regions having similar hydrophobicity characteristics, are known by those skilled in the art.

Alterations of the native amino acid sequence may be accomplished by any of a number of known techniques. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid incorporation, substitution, or deletion.

Naturally occurring MYB variants are also encompassed by the present invention. Examples of such variants are polypeptides that result from alternative mRNA splicing events or from proteolytic cleavage of the MYB proteins of the present application, wherein the MYB-binding property is retained. Alternative splicing of mRNA may yield a truncated but biologically active MYB polypeptide, such as a naturally occurring soluble form of the protein, for example. Variations attributable to proteolysis include, for example, differences in the amino or carboxyl termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the various MYB proteins.

Due to the known degeneracy of the genetic code wherein more than one codon can encode the same amino acid, a DNA sequence may vary from those presented in SEQ ID NOs: 1, 3, 5 and 7 and still encode a MYB polypeptide having the amino acid sequence set forth in SEQ ID NOs: 2, 4, 6 and 8. Such variant DNA sequences may result from silent mutations (e.g., occurring during PCR amplification), and may be the product of deliberate mutagenesis of a native sequence.

Included within the scope of the present invention, in addition to the sequences exemplified specifically herein and enumerated in the sequence listing, are cDNA sequences which are equivalent to the enumerated sequences and cDNA sequences which hybridize with the enumerated sequences and encode a polypeptide having some degree of stress-tolerance activity of the given polypeptide.

Equivalent cDNA sequences are those which encode the same polypeptide

even though they contain at least one different nucleotide from the enumerated sequence. As is known in the art, the amino acid sequence of a polypeptide is determined by the nucleotide sequence of the DNA. Because of the redundancy of the genetic code, i.e., more than one coding nucleotide triplet (codon) can be used
5 for most of the amino acids used to make proteins, different nucleotide sequences can code for a particular amino acid.

cDNA sequences that hybridize with a given enumerated sequence and encode a polypeptide or protein having at least some degree of activity of the corresponding plant stress tolerance protein are those which exhibit substantial
10 sequence identity, as defined hereinabove, with the enumerated sequence such that it hybridizes with the latter under low stringency conditions. Proteins translated from these hybridizable cDNA sequences have different primary structures from proteins translated from the enumerated sequences. However, their respective secondary structures are the same.

15

Method for Enhancing a Plant's Tolerance to Stress

The present invention also relates to methods for enhancing a plant's tolerance to stress. The method includes transforming a plant with a vector, where the vector is as described above.

20 DNA transformation may be performed using any method of plant transformation known in the art. Plant transformation methods include direct co-cultivation of plants, tissues or cells with *Agrobacterium tumefaciens* or direct infection (Miki, et al., Meth. in Plant Mol. Biol. and Biotechnology, (1993), p. 67-88); direct gene transfer into protoplasts or protoplast uptake (Paszkowski, et al., EMBO J., 12:2717
25 (1984); electroporation (Fromm, et al., Nature, 319:719 (1986); particle bombardment (Klein et al., BioTechnology, 6:559-563 (1988); injection into meristematic tissues of seedlings and plants (De LaPena, et al., Nature, 325:274-276 (1987); injection into protoplasts of cultured cells and tissues (Reich, et al., BioTechnology, 4:1001-1004 (1986)).

Such transformation may occur for example, by incorporating a recombinant vector into a plant or deleting a recombinant vector from a plant. Alternatively, the transgenic plant may be transformed by the modification of a plant with a recombinant vector. Suitable recombinant vectors are described above and plants
5 are as defined above.

Plants include the plants defined above.

**Method of Producing a Transgenic Plant
Having Enhanced Stress Tolerance**

10 Also encompassed by the present invention are methods of producing a transgenic plant having enhanced stress tolerance. The method includes transforming a plant cell or cells with a nucleic acid molecule, which encodes a MYB transcription factor. The method then includes regenerating a transgenic plant from the transformed cell(s) such that the increased expression of the MYB
15 transcription factor confers enhanced stress tolerance to the plant. Preferably, the nucleic acid sequence encoding a MYB transcription factor is operably linked to a promoter, such that the expression of the MYB polypeptide is regulated by the promoter. Preferably the nucleic acid molecule is a recombinant DNA construct.

Also, preferably in this method, the MYB transcription factor is one of
20 MYB60, MYB74, MYB75 and MYB90. In a most preferred embodiment of this aspect of the invention, the MYB transcription factor has an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

In another embodiment, the MYB transcription factor may share about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,
25 or SEQ ID NO:8. More preferably, the MYB transcription factor shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the MYB transcription factor shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

30 Plants include the plants defined above. Stress tolerance includes tolerance to various stresses including drought, salt, cold, heat and the like. The nucleic acid

sequence may be a DNA or RNA sequence and may be single stranded or double stranded.

Using the methods of the invention, transgenic plants are generated and monitored for growth. Plants exhibiting increased resistance to environmental stress, e.g., decreased susceptibility to high temperature or low temperature (chilling), drought, infection, etc., and/ or increased resistance to pathogens, are selected as superior products. These superior plants are propagated.

10 **Method of Increasing the Expression of a MYB Transcription Factor in a Plant**

The present invention also encompasses methods of increasing the expression of a MYB transcription factor in a plant. The method includes transforming a plant cell or cells with a nucleic acid molecule, which encodes a MYB transcription factor. The method then includes regenerating a transgenic plant from the transformed cell(s), such that the expression of the MYB transcription factor is increased relative to a non-transformed plant and whereby the increased expression of the MYB transcription factor confers enhanced stress tolerance to the plant. Preferably the nucleic acid molecule is a recombinant DNA construct.

Preferably in this method, the MYB transcription factor is one of MYB60, MYB74, MYB75 and MYB90. In a most preferred embodiment of this aspect of the invention, the MYB transcription factor has an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

In another embodiment, the MYB transcription factor may share about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. More preferably, the MYB transcription factor shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the MYB transcription factor shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

Plants include the plants defined above. Stress tolerance includes tolerance to various stresses including drought, salt, cold, heat and the like. The nucleic acid

sequence may be a DNA or RNA sequence and may be single stranded or double stranded.

Using the methods of the invention, transgenic plants are generated and monitored for growth. Plants exhibiting increased expression of a MYB transcription factor, as measured for example by resistance to environmental stress, e.g., decreased susceptibility to low temperature (chilling), drought, infection, etc., and/ or increased resistance to pathogens, are selected as superior products. These superior plants are propagated.

10 **Method of Increasing the Stress Tolerance of a Plant**

Further encompassed by the present invention are methods of increasing the stress tolerance of a plant. The method includes transforming a plant cell or cells with a nucleic acid sequence, which encodes a MYB transcription factor. The method then includes regenerating a transgenic plant from the transformed cell(s),
15 wherein the expression of the MYB transcription factor is increased relative to a non-transformed plant and whereby the increased expression of the MYB transcription factor confers enhanced stress tolerance to the plant, thereby increasing the stress tolerance of a plant. Preferably the nucleic acid sequence is a recombinant DNA construct.

20 Preferably in this method, the MYB transcription factor is one of MYB60, MYB74, MYB75 and MYB90. In a most preferred embodiment of this aspect of the invention, the MYB transcription factor has an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

In another embodiment, the MYB transcription factor may share about 50%
25 to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. More preferably, the MYB transcription factor shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the MYB transcription factor shares about 70% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,
30 or SEQ ID NO:8.

Plants include the plants defined above. Stress tolerance includes tolerance to various stresses including drought, salt, cold, heat and the like. The nucleic acid sequence may be a DNA or RNA sequence and may be single stranded or double stranded.

- 5 Using the methods of the invention, transgenic plants are generated and monitored for growth. Plants exhibiting increased resistance to environmental stress, e.g., decreased susceptibility to low temperature (chilling), drought, infection, etc., and/ or increased resistance to pathogens, are selected as superior products. These superior plants are propagated.

10

Method for Enhancing a Plant's Sensitivity to Stress

- The present invention also relates to methods for enhancing a plant's sensitivity to stress. The method includes transforming a plant with a vector encoding a polynucleotide sequence that is complementary to SEQ ID NO:1, SEQ
15 ID NO:3, SEQ ID NO:5 or SEQ ID NO:7, or to the mRNA encoded by SEQ ID NO:1 SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7, where the vector is as described above. Suitable methods of transformation are described above.

Plants include the plants defined above.

20

Method of Producing a Transgenic Plant with Enhanced Stress Sensitivity

- The present invention is further directed to a method of producing a transgenic plant having enhanced stress sensitivity. Such a plant preferably has a reduced level of MYB transcription factors, preferably MYB60, MYB74, MYB75
25 and MYB90 as compared to an unmodified plant. The method includes (1) transforming a plant with a vector, specifically a recombinant vector from any of the antisense nucleic acid molecules, as described above; (2) allowing the plant to grow to at least a plantlet stage; (3) assaying the transformed plant or plantlet for altered MYB activity and/or environmental stress sensitivity; and (4) selecting and growing

a plant having altered MYB activity and/or environmental stress sensitivity compared to a non-transformed plant.

The plants of this method are as described above. Preferably, the plant may be used as an environmental monitor.

5

A Transformed Transgenic Plant

The present invention further relates to a transgenic plant that is stably transformed. The transgenic plant is preferably stably transformed with a MYB gene or variant thereof, which is expressed so as to enhance stress tolerance in the
10 plant. The DNA may further comprise a screenable marker gene. Alternatively, the transgenic plant may be transformed by an antisense gene.

Also encompassed by the present invention are seeds transformed with a MYB gene or functional derivative or variant thereof. The seed may be transformed by the incorporation, deletion or modification of a seed, plant, plant part or progeny
15 thereof with a recombinant vector as described herein. Such recombinant vectors may be from any of the nucleic acid molecules or antisense nucleic acid molecules described herein.

Particular benefits may be realized by the transformation of plant cells or seeds with any of the nucleic acids comprising the genes described herein or variants
20 thereof. (That is, by incorporation, deletion or modification of these nucleic acids into a plant or seed).

Various methods for accomplishing the genetic transformation of plants (that is, stably introducing foreign DNA into plant) are known in the art. Suitable methods are believed to include virtually any method by which DNA can be
25 introduced into a cell, such as by *Agrobacterium* infection or direct delivery of DNA such as, for example, by PEG-mediated transformation, by electroporation or by acceleration of DNA coated particles, etc. Acceleration methods are generally preferred and include, for example, microprojectile bombardment and the like.

In the microprojectile bombardment method, non-biological particles may be

coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum, and the like.

Transgenic plants made in accordance with the present invention may be prepared by nucleic acid transformation using any method of plant transformation
5 known in the art.

Generally a complete plant is ultimately obtained from the transformation process. Plants are regenerated from protoplasts, callus, tissue parts or explants, etc. Plant parts obtained from the regenerated plants, such as leaves, flowers, fruit, seeds and the like are included in the definition of "plant" as used herein. Progeny,
10 variants and mutants of the regenerated plants are also included in the definition of "plant."

The transformation or genetic modification can effect a permanent change in the MYB levels in the plant and be propagated in offspring plants by selfing or other reproductive schemes. The genetically altered plant may be used to produce a new
15 variety or line of plants wherein the alteration is stably transmitted from generation to generation.

Method of Screening a Plant for Stress Tolerance

Also encompassed by the present invention is a method of screening a plant
20 for stress tolerance. The method includes screening the expression level of a stress tolerance-related MYB polypeptide in a plant. The plants of this method are as described above.

After delivering nucleic acids, or variants thereof to recipient cells by any of the methods discussed above, the transformed cells may be identified for further
25 culturing and plant regeneration. In this method, the transformed cell or plant is selected or screened by conventional techniques. This step may include assaying cultures directly for a screenable trait or by exposing the bombarded cultures to a selective agent or agents.

In order to improve the ability to identify transformants, one may desire to
30 employ a selectable or screenable marker gene as, or in addition to, the expressible

gene of interest. Marker genes code for phenotypes that allow cells that express the marker gene to be distinguished from cells that do not have the marker. Such genes may encode either a selectable or screenable marker, depending on whether the marker confers a trait which one can select for by chemical means, i.e., through the use of a selective agent (e.g., an herbicide, or the like), or whether it is simply a trait that one can identify through observation or testing. Examples of suitable marker genes are known to the art and can be employed in the practice of the invention. For example, suitable markers may include markers that encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes which can be detected catalytically. Secretable proteins fall into a number of classes, including small, diffusible proteins detectable, e.g., by ELISA, small active enzymes detectable in extracellular solution (e.g., α -amylase, β -lactamase, phosphinothricin transferase), or proteins which are inserted or trapped in the cell wall (such as proteins which include a leader sequence such as that found in the expression unit of extensin or tobacco PRS), of course, in light of this disclosure, numerous other possible selectable and/or screenable marker genes will be apparent to those of skill in the art. Therefore, the foregoing discussion is intended to be exemplary rather than exhaustive.

The transformed cell or plant contains the chimeric DNA sequence and is then regenerated, if desired, by known procedures, for both monocot and dicot plants. The regenerated plants are screened for transformation by standard methods. Progeny of the regenerated plants is continuously screened and selected for the continued presence of the integrated DNA sequence in order to develop improved plant and seed lines. The DNA sequence can be moved into other genetic lines by a variety of techniques, including classical breeding, protoplast fusion, nuclear transfer and chromosome transfer.

Where both an expressible gene that is not necessarily a marker gene is employed in combination with a marker gene, one may employ the separate genes on either the same or different DNA segments for transformation. In the latter case,

the different vectors are delivered concurrently to recipient cells to maximize co-transformation.

In order for a newly inserted gene or DNA sequence to be expressed, resulting in production of the protein which it encodes, or in the case of antisense DNA, to be transcribed, resulting in an antisense RNA molecule, the proper regulatory elements should be present in proper location and orientation with respect to the gene or DNA sequence. The regulatory regions may include a promoter, a 5'-non-translated leader sequence and a 3'-polyadenylation sequence as well as enhancers and other regulatory sequences.

Promoter regulatory elements that are useful in combination with the MYB gene to generate sense or antisense transcripts of the gene include any effective promoter in general, and more particularly, a constitutive promoter such as the fig wart mosaic virus 35S promoter, the cauliflower mosaic virus promoter, CaMV35S promoter, or the MAS promoter, or a tissue-specific or senescence-induced promoter, such as the carnation petal GST1 promoter or the *Arabidopsis* SAG12 promoter (See, for example, J.C. Palaqui et al., *Plant Physiol.*, 112:1447-1456 (1996); Morton et al., *Molecular Breeding*, 1:123-132 (1995); Fobert et al., *Plant Journal*, 6:567-577 (1994); and Gan et al., *Plant Physiol.*, 113:313 (1997), incorporated herein by reference). Preferably, the promoter used in the present invention is a constitutive promoter.

Expression levels from a promoter which is useful for the present invention can be tested using conventional expression systems, for example by measuring levels of a reporter gene product, e.g., protein or mRNA in extracts of the leaves, flowers, fruit or other tissues of a transgenic plant into which the promoter/reporter have been introduced.

Method for Increasing the Stress Resistance of a Crop in a Field

Another embodiment of the invention is a method for increasing the stress resistance of a crop in a field. The method includes planting in the field seeds or plants, such as the transgenic plants or seeds described herein, which are

transformed with the vectors described herein, by any of the methods described herein. Suitable methods of planting are known to those in the art.

Method of Inhibiting the Expression of MYB Genes in a Plant

5 The present invention further relates to a method of inhibiting the expression of MYB genes in a plant cell, the method includes integrating into the genome of a plant a vector specifically, a recombinant vector from any of the antisense nucleic acid molecules, as described above, and growing the plant. In this method, the antisense nucleic acid molecule is transcribed, such that expression of the MYB
10 gene is inhibited.

Method of Assaying the Environmental Conditions of a Field

 The invention further relates to a method of assaying the environmental conditions of a field. Such a method includes planting any of the plants described
15 herein, including those transformed by the vectors described herein, both antisense and non-antisense, and monitoring the growth of the plant.

Method of Increasing the Production of Products of the Phenylpropanoid Biosynthesis Pathway in a Plant

20 Also encompassed by the present invention is a method of increasing the production of products of the phenylpropanoid biosynthesis pathway in a plant. The method includes transforming a plant cell with a recombinant DNA construct comprising a nucleic acid sequence encoding a MYB transcription factor. The method then includes regenerating a transgenic plant from the transformed cell,
25 wherein the expression of the MYB transcription factor increases the expression of genes encoding gene products affecting the phenylpropanoid pathway, thereby increasing the production of products of the phenylpropanoid biosynthesis pathway.

 Products of the phenylpropanoid pathway include, but are not limited to stilbenes, flavonoids, lignins, salicylic acid, anthocyanins, phenolic derivatives and
30 the like.

Preferably in this method, the MYB transcription factor is one of MYB60, MYB74, MYB75 and MYB90. In a most preferred embodiment of this aspect of the invention, the MYB transcription factor has an amino acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

5 In another embodiment, the MYB transcription factor may share about 50% to about 100% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. More preferably, the MYB transcription factor shares about 65% to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. Most preferably, the MYB transcription factor shares about 70%
10 to about 99% sequence identity with SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

Plants include the plants defined above. Stress tolerance includes tolerance to various stresses including drought, salt, cold, heat and the like. The nucleic acid sequence may be a DNA or RNA sequence and may be single stranded or double
15 stranded.

Using the methods of the invention, transgenic plants are generated and monitored for growth. Plants exhibiting increased expression of products of the phenylpropanoid biosynthesis pathway, as exhibited for example by decreased susceptibility to high temperature or low temperature (chilling), drought, infection,
20 etc., and/ or increased resistance to pathogens, are selected as superior products. These superior plants are propagated.

Further encompassed by the present invention is a method of decreasing the production of products of the phenylpropanoid biosynthesis pathway in a plant. The method includes (i) transforming a plant cell with a vector comprising an antisense
25 nucleic acid molecule substantially complementary to at least a portion of a DNA molecule encoding a MYB transcription factor or at least a portion of an RNA sequence encoded by the DNA molecule encoding said MYB transcription factor; and regulatory sequences operatively linked to the antisense nucleic acid molecule; such that the nucleic acid molecule is expressed in a plant cell into which it is
30 transformed; and (ii) growing the plant, whereby the antisense nucleic acid molecule

is transcribed, such that expression of the MYB gene is inhibited, thereby decreasing the production of products of the phenylpropanoid biosynthesis pathway. For example, products of the phenylpropanoid pathway such as stilbenes, flavonoids, lignins, salicylic acid, anthocyanins, phenolic derivatives and the like are decreased
5 by this method.

In the case of antisense, transgenic plants are generated and monitored for growth. Plants exhibiting an increased stress sensitivity are selected and propagated.

10 **Isolation of MYB Nucleic Acids and Construction of MYB Encoding Vectors**

Nucleic acid molecules encoding the MYB transcription factors of the present invention may be prepared by two general methods: (1) they may be synthesized from appropriate nucleotide triphosphates, or (2) they may be isolated from biological sources. Both of the above methods are well known in the art.

15 Nucleic acid sequences encoding the MYB transcription factors of the present invention may be isolated from appropriate biological sources using methods known in the art. In accordance with the present invention, nucleic acids having the appropriate level of sequence identity with the protein coding region of SEQ ID NOs: 1, 2, 4, or 5 may be identified by using hybridization and washing conditions of
20 appropriate stringency. For example, hybridizations may be performed, according to the method of Sambrook et al., (22) using a hybridization solution including: 5 times SSC, 5 times Denhardt's reagent, 1.0% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA, 0.05% sodium pyrophosphate and up to 50% formamide. Hybridization is carried out at 37-42°C for at least six hours. Following
25 hybridization, filters are washed as follows: (1) 5 minutes at room temperature in 2 times SSC and 1% SDS; (2) 15 minutes at room temperature in 2 times SSC and 0.1% SDS; (3) 30 minutes-1 hour at 37° C. in 1 times SSC and 1% SDS; (4) 2 hours at 42-65°C in 1 times SSC and 1% SDS, changing the solution every 30 minutes.

Nucleic acids of the present invention may be maintained as DNA in any
30 convenient cloning vector. In a preferred embodiment, clones are maintained in

plasmid cloning/expression vector, such as pBluescript (Stratagene, La Jolla, Calif.), which is propagated in a suitable *E. coli* host cell.

A full-length MYB polypeptide of the present invention may be prepared in a variety of ways, according to known methods. The protein may be purified from
5 appropriate sources, e.g., plant or animal cultured cells or tissues, by immunoaffinity purification.

Alternatively, according to a preferred embodiment, larger quantities of MYB polypeptide may be produced by expression in a suitable procaryotic or eucaryotic system. For example, part or all of a DNA molecule, such as the cDNA
10 having SEQ ID NO: 1, may be inserted into a plasmid vector adapted for expression in a bacterial cell, such as *E. coli*, or into a baculovirus vector for expression in an insect cell. Such vectors comprise the regulatory elements necessary for expression of the DNA in the host cell (e.g. *E. coli*, plant cell or insect cell), positioned in such a manner as to permit expression of the DNA in the host cell. Such regulatory
15 elements required for expression include promoter sequences, transcription initiation sequences and, optionally, enhancer sequences.

The MYB polypeptide produced by gene expression in a recombinant procaryotic or eucaryotic system may be purified according to methods known in the art. In a preferred embodiment, a commercially available expression/secretion
20 system can be used, whereby the recombinant protein is expressed and thereafter secreted from the host cell, to be easily purified from the surrounding medium. If expression/secretion vectors are not used, an alternative approach involves purifying the recombinant protein by affinity separation, such as by immunological interaction with antibodies that bind specifically to the recombinant protein or nickel columns
25 for isolation of recombinant proteins tagged with 6-8 histidine residues at their N-terminus or C-terminus. Such methods are commonly used by skilled practitioners.

The MYB proteins of the invention may be analyzed according to standard procedures. For example, such proteins may be subjected to amino acid sequence analysis, according to known methods.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting to the present invention.

5 EXAMPLE 1

Genes induced during water stress conditions are not only thought to protect cells from osmotic stress but also to be involved in the regulation of genes for signal transduction in water deficit response. A first group of genes codes for proteins that directly function in stress tolerance. A second group of genes induced under water
10 stress conditions encodes for regulatory proteins that function in signal transduction pathways. Examples are protein kinases, PLC, 14-3-3 proteins and transcription factors directly involved in the further control of gene expression during water stress response. Certain genes respond to drought, salt and cold stress at the transcriptional level. Expression patterns of dehydration-inducible genes are complex: some genes
15 respond to water stress very rapidly, whereas others are induced slowly after the accumulation of ABA. Most of the genes that respond to drought, salt and cold stress can also be induced by exogenous applications of ABA. It is believed that dehydration may trigger the production of ABA, which in turn induces various genes. On the other hand, several genes that are induced by water stress are not
20 responsive to exogenous ABA treatment. Analysis of the expression of water-stress-inducible genes by ABA in *ABA-deficient (aba)* or *ABA-insensitive (abi)* *Arabidopsis* mutants have indicated that some of the stress-inducible genes do not require an accumulation of endogenous ABA under drought or cold conditions. These observations suggest the existence of both ABA-independent and ABA-
25 dependent signal transduction cascades between the initial signal of drought or cold and the further expression of specific genes. In addition, analysis of the expression of ABA-inducible genes revealed that several of them require protein biosynthesis for their ABA induction, while others do not, suggesting the existence of at least two independent pathways between the upstream production of endogenous ABA and
30 gene expression during stress. The ABA-inducible genes that do not require protein

biosynthesis for their expression contain a potential ABA-responsive-element, termed ABRE (PyACGTTCCG) (SEQ ID NO:9) in their promoter regions. The ABRE resembles the G-box element, an ACGT "core" containing element, that functions in the regulation of plant genes in a variety of environmental conditions, such as light, UV, wounding and anaerobiosis. Basic region leucine zipper (bZIP) proteins have been shown to be involved in the binding to this class of elements. Furthermore, a coupling element is required to specify the function of the ABRE, constituting an ABA-responsive complex.

Along the second ABA-dependent pathway, protein biosynthesis is necessary for the expression of water-stress-inducible genes. A 67 bp region in the promoter of *rd22*, an *Arabidopsis* gene whose expression is mediated by ABA and requires protein biosynthesis, is essential and sufficient for its dehydration and ABA-inductibility. This region contains two closely located putative recognition sites for the basic helix-loop-helix protein MYC (CANNTG) (SEQ ID NO:10) and one for a MYB protein (PyAACPyPu) (SEQ ID NO:11). However, this region does not contain ABRE sequences.

A possible role of the *Arabidopsis* AtMYB2 in water stress response is the induction of *rd22* gene and under low oxygen conditions the induction of the *ADHI*. *Rd22BP1* gene, which encodes a MYC transcription factor, and *AtMYB2* are both induced by dehydration stress. The corresponding proteins bind *in vitro* to have the 67 bp region of the *rd22* gene promoter. These results suggest that MYB and MYC homologues are involved in the regulation of gene expression along one of the ABA-dependent signal cascade. However, the existence of several genes induced by drought and cold in *aha* and *abi* *Arabidopsis* mutants suggests the presence of signal transduction pathways that do not require ABA accumulation for their induction. A 9 bp dehydration responsive element, termed DRE (TACCGACAT) (SEQ ID NO:12) is essential for the ABA-independent induction of many stress-inducible genes such as *rd29A*, *kin1*, *cor6.6* and *rd17*, under drought, high salt and high and low temperature conditions. Concerning the ABA-independent pathways, the existence of several drought-induced genes that do not respond to cold or ABA

treatment suggest that there is at least a fourth pathway, which can be involved in the plant tolerance to environmental stress, such as dehydration. As suggested by the classes of mutants recovered that respond in different ways to ABA, cold and osmotic stress ABA-dependent and ABA-independent pathways may interact and
5 coverage to activate stress genes.

Several MYB recognition sites have been found in the promoter regions of different genes induced under osmotic stresses. Therefore, Applicants studied the possible involvement of MYB proteins in the regulation of stress induced genes. The expression pattern of four MYB genes were analyzed in response to different
10 osmotic stress. Certain putative target genes known to be induced by water stress, i.e., *AtP5CS1*, *rd22*, *erd10* and *ADH1*, were also included in this analysis to hypothetically localize these MYB transcription factors along the four different signal transduction pathways recently proposed.

Seeds of wild type *Arabidopsis thaliana* (Columbia ecotype) were used in
15 this study. For cold treatment seeds were sown on Einhietserde soil, treated at 4°C for 4 days to promote even germination, then grown with a 16-hours light/8-hours dark cycle at 22°C for 4 weeks and subsequently incubated at 4°C for up to 48 hours in the dark. The entire aerial part of the plants was collected after 2, 4, 6, 8, 24, and 48 hours.

20 For drought, ABA, PEG and NaCl treatments seeds were surface-sterilized with ethanol for 2 minutes, then with a solution of sodium hypochlorite (0.5% v/v) for 5 minutes, rinsed 3 times with sterilized distilled water, treated at 4°C for 4 days to promote even germination. For drought treatment sterilized seeds were sown on MS medium agar (0.8% w/v) plates, supplemented with sucrose (1% w/v) and MES (0.5
25 g L⁻¹), grown with a 16-hour light/8-hour dark cycle at 22°C for 2 weeks, then dehydrated on 3MM paper at 22°C in the light for 1, 2, 3, 5, and 7 hours.

For ABA, PEG (Polyethyleneglycol 6000) and NaCl treatments plants were grown in liquid MS medium, supplemented with sucrose (3% w/v) and MES (0.5 g L⁻¹), with a 16-hour light/8-hour dark cycle at 22°C for 3 weeks in an orbital shaker,
30 then ABA (\pm cis-trans isomers) or PEG 6000 or NaCl were added at a final

concentration of 100 μ M, 30% w/v and 200 mM respectively; the samples were collected after 1, 2, 4, 6, 8, 16, 24, and 48 hours. For PEG 30% treatment after 6 hours of stress samples were re-hydrated transferring the plants in fresh medium without PEG and collected after 1, 4 and 24 hours (R1h, R4h and R24h). An untreated culture (PEG and NaCl control) and a culture treated with the solvent ethanol used for the ABA treatment (ABA control) were also harvested. In each case the plants were subjected to the stress treatments for various time periods, frozen in liquid nitrogen and stored at -80°C.

The results are shown in Table 1.

RNA extraction and RT-PCR analysis

Total RNA was isolated from whole plants collected at various time periods of treatments by methods known to those in the art.

Reverse transcriptase polymerase chain reaction (RT -PCR) was used to detect *AtMYB75* and *AtP5CS1* genes transcripts. All RNA samples were treated with DNaseI (15 units) before cDNA synthesis. First strand cDNA synthesis was carried out from 6 μ g of total RNA with an oligo (dT) and RT Superscript™ II (300 units) as recommended by the manufacturer. The primer used was a 35-base oligonucleotide with 17dT residues and an adapter (5'-GGGAATTCGTCGACAAGC-3') (SEQ ID NO:13) sequence. First-strand cDNA was used as template for PCR amplification. Amplification reactions containing an aliquot of cDNA, 1X PCR Buffer II, 2.5 mM MgCl₂, 200 μ M of each dATP, dCTP, dGTP and dTTP, 0.1 μ M of each primer and 2.5 unit of AmpliTaq were performed in a final volume of 50 μ l. After the first denaturation step (2 min and 30 sec at 94°C), the reaction mix underwent 20 cycles of denaturation at 94°C at 45 sec, annealing at 55°C for 1 min and extension at 72°C for 2 min. A final extension at 72°C for 7 min was performed to complete the reaction. A set of primers specific for the *TSB1* gene of *Arabidopsis*, which encodes the β subunit of tryptophan synthase, were used to standardize the concentration of the different samples. The length of the amplified product was 476 bp. To ensure the amplification reactions

were within linear ranges, the reactions were carried out for 20 cycles. The PCR products were fractionated on 2% w/v agarose gels and transferred onto Hybond N+ nylon membranes and hybridized employing probe labeled with fluorescein, according to the manufacturers' protocols. This standardization was confirmed using a set of primers specific for *Arabidopsis ACT1* gene that encodes for actin. For mRNA detection of the genes under analysis, the specific primer sets were used. The PCR products were then separated on agarose gels 2% w/v, and transferred onto Hybond N+ nylon membranes and hybridized with random primed fluorescein fragments.

Four MYB genes were studied in response to osmotic stress. Quantitative RT-PCR analysis was performed on RNA samples obtained from plants exposed to abiotic stresses like low temperature, drought, high salt, PEG and abscisic acid supply. Applicants analyzed the kinetics of expression of four MYB genes and of four putative target genes known to be induced by water stress, *AtP5CS1*, *rd22*, *erd10* and *ADH1*. The cDNA and amino acid sequences of *AtMYB60* are reported in Figure 1, of *AtMYB74* in Figure 2, of *AtMYB75* in Figure 3A and Figure 3B, and of *AtMYB90* in Figure 4.

Table 1 summarizes the results obtained for all genes analyzed.

Table 1

Schematic representation of MYB genes and *AtP5CS1*, *erd10*, *rd22* and *ADH1* genes expression under different stress conditions: drought, PEG (30%), ABA (100 μ M), NaCl (200 mM) and cold (4°C).

		Drought	PEG	ABA	NaCl	Cold
25	<i>AtMYB60</i>	---	-	---	-	0
	<i>AtMYB74</i>	+++	+++	+++	0	+
	<i>AtMYB75</i>	+++	+++	+++	+++	n.d.
	<i>AtMYB90</i>	+	0	+++	+++	n.s.
30	<i>AtP5CS1</i>	+	+++	+	0	+++
	<i>erd10</i>	+++	+++	+	+	+++

rd22	+	+	+	+	+
ADH1	+	+	+	0	+

Symbols indicated: n.s. no signal; n.d. not determined; 0 no induction; + slight induction; + + + high induction; - slight repression; - - - high repression

The expression of *AtP5CS1* (see Figure 5) was strongly induced within 1 hour after the initiation of drought treatment and high levels of transcript were maintained during 7 hours of dehydration. These data are consistent with previously published results where the expression of *AtP5CS1* was induced by exogenous ABA treatment. ABA supply induced *AtP5CS1* gene expression within 2 hours, reaching a peak after 4 hours, then the level of this transcript decreased gradually. In PEG treated plants *AtP5CS1* mRNA was induced in a two-phase time course: the first peak of induction was observed after 1 hour of PEG supply, then the level of expression decreased; after 6 hours mRNA accumulated once again. When plants treated with PEG for 6 hours were re-hydrated for 1, 4 and 24 hours the level of the transcript decreased gradually, returning to the level present in untreated plants. A similar two phases induction process was also observed for *rd22* (see Figure 6), *erd10* (see Figure 7) and *ADH1* (see Figure 8). The same kinetics had been previously reported for *erd10* gene upon cold stress and for *rd29A* during dehydration treatment. It has been shown that in dehydration conditions endogenous ABA began to accumulate 2 h after the beginning of the treatment and reached its maximum at 10 hours. Taken together, these results suggest that the first rapid induction in the two-step kinetics is not mediated by ABA, while the late induction is ABA-dependent. Our results from PEG and ABA treatments confirm a role for ABA in the late induction not only for *erd10* but also for *AtP5CS1*, *rd22*, and *ADH1* while early transcript accumulation seems to be ABA-independent.

Among the MYB genes analyzed only *AtMYB74* (see Figure 9) and *AtMYB75* (see Figure 10) are rapidly induced in response to PEG 30% and their transcripts are maintained at high level throughout the length of the treatments even if only *AtMYB74* shows a clear two phases induction process. Their transcripts are

also induced very rapidly by exogenous ABA and drought treatments, while their expression is differentially modulated by NaCl treatment (Table 1). Therefore, *AtMYB74* and *AtMYB75* are believed to be good candidates to regulate genes involved in water stress response along two different signal transduction pathways an ABA-independent pathway, early activated under osmotic stress and responsible for the rapid induction of *AtMYB74* and *AtMYB75* as well as an ABA-dependent pathway, activated after ABA accumulation and responsible for the second phase of induction of those genes. See the comparison of the expression patterns of *AtMYB75* and *AtMYB74* to ER10, ADH1, P5CS1 and RD22 in Figure 11.

Another gene, *AtMYB90*, phylogenetically correlated to *AtMYB75*, showed a similar pattern of expression in response to ABA and NaCl, while it is not induced by PEG treatment (see results of the RT-PCR analysis in Figure 12). In response to drought treatment *AtMYB90* transcript appears three hours after the beginning of the stress and its level is reduced with respect to that of *AtMYB74* and *AtMYB75* (Table 1). Therefore, its role in stress response is not very clear. *AtMYB60* was the only *MYB* gene analyzed that was repressed by water and osmotic stress conditions (Table 1): its transcript levels decreased significantly within 1 hour after the initiation of drought and ABA treatment and only slightly after PEG and NaCl supply (between 4 and 6 hours after the initiation of the treatments).

It is believed from these experiments that *AtMYB74* is activated by stress both by an ABA dependent and an ABA independent pathway, while *AtMYB60* is repressed by stress (particularly drought stress) in an ABA dependent manner.

EXAMPLE 2

Phenylpropanoid molecules comprise a set of important secondary products such as anthocyanin pigments, flavonoids, phytoalexins, phenolics acids which are involved in the protection of plants against UV damage, oxidative stress, pathogen attack, etc. The biochemical pathways leading to the synthesis of most of these

compounds are understood and several of the structural and regulatory genes involved have been cloned from maize, petunia and snapdragon.

In maize the enzymes involved in this biosynthesis are regulated in a coordinated way as a result of the activation of regulatory genes that are expressed in a tissue specific manner. Genetic and molecular analyses indicate that the regulatory genes can be grouped in two families, the *R/B* gene family, which encodes related proteins with a basic-helix-loop-helix (bHLH) DNA binding domain and the *CI/PI* family, which encodes related proteins with MYB-domain. A member of each of the two families is preferably expressed for the transcriptional activation of the biosynthetic genes.

The RNA gel blot and RT-PCR analysis has revealed that light dependent anthocyanin accumulation is due to the light induced expression of the MYB *CI* and *PI* genes.

In *Arabidopsis* the structural genes of the flavonoid pathway had previously been studied and cloned but the transcription factors regulating this biosynthesis were not previously understood. To understand the role of MYB genes in *Arabidopsis*, Applicants undertook a quantitative RT-PCR analysis performed on RNA samples of *Arabidopsis* obtained from several tissues and at different times after treatment with radiations such as white and blue light, UVA, UVB. The expression patterns were then compared with those of chalcone synthase (CHS) gene and dihydroflavonol-4-reductase (D4R) gene, structural genes of the flavonoid pathway. The expression patterns are shown in Fig. 15. The MYB-75 and MYB-90 expression patterns in response to white, blue, UV-A and UV-B light are consistent with their putative role in the control of phenylpropanoid pathway.

The expression pattern of AtMYB75 and chalcone synthase (CHS gene), induced by white, blue, UVA and UVB light suggests that MYB 75 could regulate the expression of CHS while that of AtMYB90 and D4R induced with a similar kinetic by white, blue and UVA light suggests that MYB90 could regulate the expression of D4R. The believed roles of MYB75 and MYB90 in the phenylpropanoid metabolic pathway are depicted in Figure 16.

Among the more than 100 MYB genes cloned in *Arabidopsis*, the ones showing the highest similarity to the maize *C1* and *Pl* gene are AtMYB75 and AtMYB90. Thus, Applicants believe that AtMYB75 and AtMYB90 are transcription factors that regulate flavonoid biosynthesis.

5

EXAMPLE 3

Construction and Analysis of MYB Transgenic plants.

Individually, each of the MYB genes of the present invention are placed in a sense or antisense orientation under the control of the constitutive CaMV 35 S promoter and are introduced into the tobacco cultivar Xanthi. Independent transgenic tobacco plants are generated. Vector-only transformed plants are also generated to be used as controls. The MYB transgenic plants exhibit enhanced resistance to environmental stresses as compared to the control plants.

Transgenic plants having MYB genes introduced in the antisense orientation exhibit increased sensitivity to environmental stresses, such as drought and high salt conditions, as compared to the control plants. These plants show a decrease in expression of the MYB genes that enhance resistance to the high stress conditions.

Progeny of plants having the MYB introduced in the sense and antisense orientation are each collected and further analyzed. Resistant and sensitive progeny are generated for further use.

EXAMPLE 4

To induce anthocyanin pigmentation in maize, the contemporary expression of one member of the R/Sn gene family (bHLH transcription factors) and a member of the C1/Pl gene family (MYB) is needed. So we used a maize line carrying deletion for the *r* genes and carrying a small *pl* gene (recessive allele). In this condition no anthocyanin pigment are produced.

Applicants performed a shot gun experiment. The constructs used were cDNA of the different genes under 35S promoter. Individually, MYB75 and MYB90 genes from *Arabidopsis*, were introduced into maize mutants lacking

anthocyanin. Applicants shot gun germinated maize seeds with the different combinations of construct and after four days we scored the seedlings for red spots. The presence of red spots is due to an accumulation of anthocyanin, which indicates that the transcription factors were expressed and able to induce the transcription of the structural genes of the anthocyanin biosynthetic pathway. The results of this experiment are set forth in Table 2 below

TABLE 2

	<u>Constructs used</u>	<u>observation</u>
10	Sn + C1	red pigmented cells
	Sn only	no pigmented cells
	C1 only	no pigmented cells
	Sn + MYB 75	red pigmented cells
	Sn + MYB90	red pigmented cells
15	Sn + MYB75 + MYB 90	red pigmented cells

These results in Table 2 indicate that AtMYB75 and AtMYB90 are able to complement maize mutants and are able to functional substitute the maize C1 gene in activating the anthocyanin biosynthesis in maize. Thus, anthocyanin was induced in the maize plants. This experiment shows that MYB75 and MYB90 genes cure the defect of a lack of anthocyanin in mutant plants. The experiment also show that the MYB genes may be stably transformed into plants and that cross species introduction of these genes is successful. Applicants believe that the MYB75 and MYB90 genes activate the anthocyanin pathway.

25

Conclusion

Although the present invention has been described with respect to exemplary embodiments, there are many other variations of the above-described embodiments which will be apparent to those skilled in the art, even where elements have not

explicitly been designated as exemplary. It is understood that these modifications are within the teaching of the present invention.

We claim:

1. A compound comprising a nucleic acid molecule comprising a sequence that encodes a plant stress tolerance-related myloblastosis (MYB) transcription factor.
- 5 2. The compound of claim 1, wherein the MYB transcription factor is selected from the group consisting of MYB60, MYB74, MYB75, and MYB90.
3. The compound of claim 1, wherein the nucleic acid molecule encodes
10 an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
4. The compound of claim 1, wherein the nucleic acid molecule encodes an amino acid sequence having a sequence identity of about 50% to about 100%
15 with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
5. The compound of claim 1, wherein the nucleic acid molecule encodes an amino acid sequence having a sequence identity of about 65% to about 99% with
20 an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
6. The compound of claim 1, wherein the nucleic acid molecule encodes an amino acid sequence having a sequence identity of about 70% to about 99% with
25 an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.
7. The compound of claim 1, wherein the nucleic acid sequence is a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ
30 ID NO:5, and SEQ ID NO:7.

8. The compound of claim 1, wherein the nucleic acid sequence is a sequence sharing a sequence identity of about 50% to about 100% with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

5

9. The compound of claim 1, wherein the nucleic acid sequence is a sequence sharing a sequence identity of about 65% to about 99% with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

10

10. The compound of claim 1, wherein the nucleic acid sequence is a sequence sharing a sequence identity of about 70% to about 99% with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7.

15

11. A recombinant vector comprising the nucleic acid molecule of claim 1.

12. A compound comprising a nucleic acid molecule comprising a nucleic acid molecule encoding an RNA molecule which is substantially homologous to at least a portion of an RNA transcript of a plant MYB gene, wherein said plant MYB gene hybridizes under low stringency conditions with a nucleic acid sequence encoding a MYB transcription factor selected from the group consisting of MYB60, MYB74, MYB75, and MYB90.

25

13. The compound of claim 12, wherein the MYB transcription factor is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

14. The compound of claim 12, wherein the MYB transcription factor has a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

5

15. The compound of claim 12, wherein the MYB transcription factor has a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

10

16. The compound of claim 12, wherein the MYB transcription factor has a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

15

17. The compound of claim 12, wherein the MYB gene has a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

20

18. The compound of claim 12, wherein the MYB gene has a sequence identity of about 50% to about 100% with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

25

19. The compound of claim 12, wherein the MYB gene has a sequence identity of about 65% to about 99% with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

20. The compound of claim 12, wherein the MYB gene has a sequence identity of about 70% to about 99% with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

5

21. The compound of claim 12, wherein the nucleic acid molecule comprises at least about six nucleotides.

22. A recombinant vector for transformation of plant cells, comprising
10 a nucleic acid molecule substantially homologous to (1) at least a portion of a DNA molecule encoding a MYB transcription factor selected from the group consisting of MYB60, MYB74, MYB75 and MYB90, or (2) at least a portion of an RNA sequence encoded by the DNA molecule encoding said MYB transcription factor; and

15 regulatory sequences operatively linked to the nucleic acid molecule such that the nucleic acid molecule is expressed in a plant cell into which it is transformed.

23. A MYB polypeptide comprising a plant stress tolerance-related MYB
20 transcription factor.

24. The MYB polypeptide of claim 23, wherein the MYB polypeptide is a MYB transcription factor having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO: 6, and SEQ ID NO:8.

25

25. The MYB polypeptide of claim 23, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

30

26. The MYB polypeptide of claim 23, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

5

27. The MYB polypeptide of claim 23, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

10

28. An antibody of the MYB polypeptide of claim 23.

29. The antibody of claim 28, wherein the MYB polypeptide is a MYB transcription factor having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

15

30. The antibody of claim 28, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

20

31. The antibody of claim 28, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8.

25

32. The antibody of claim 28, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

30

33. A variant of the MYB polypeptide of claim 23.

34. The variant of claim 33, wherein the MYB polypeptide is a MYB transcription factor having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

35. The variant of claim 33, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

36. The variant of claim 33, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

37. The variant of claim 33, wherein the MYB polypeptide is a MYB transcription factor sharing a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

38. A method for enhancing a plant's tolerance to stress comprising transforming said plant with a vector as claimed in claim 11.

39. The method of claim 38, wherein said plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

40. A method of producing a transgenic plant with enhanced stress tolerance comprising:

transforming at least one plant cell with a recombinant DNA construct comprising a nucleic acid sequence encoding a MYB transcription factor; and regenerating a transgenic plant from the transformed cell, whereby the increased expression of the MYB transcription factor confers enhanced stress
5 tolerance to the plant.

41. The method of claim 40, wherein said MYB transcription factor is selected from the group consisting of MYB60, MYB74, MYB75 and MYB90.

10 42. The method of claim 40, wherein said MYB transcription factor has a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

43. The method of claim 40, wherein the MYB transcription factor shares
15 a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

44. The method of claim 40, wherein the MYB transcription factor shares
20 a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

45. The method of claim 40, wherein the MYB transcription factor shares
25 a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7.

46. The method of claim 40, wherein the nucleic acid sequence encoding
30 a MYB transcription factor is operatively linked to a promoter.

47. The method of claim 40, wherein the stress tolerance comprises salt stress tolerance.

48. The method of claim 40, wherein the stress tolerance comprises
5 drought stress tolerance.

49. The method of claim 40, wherein the stress tolerance comprises cold stress tolerance.

10 50. The method of claim 40, wherein the stress tolerance comprises heat stress tolerance.

51. The method of claim 40, wherein the nucleic acid sequence is a DNA sequence.
15

52. The method of claim 40, wherein the nucleic acid sequence is an RNA sequence.

52. The method of claim 40, wherein the nucleic acid sequence is a single
20 stranded sequence.

52. The method of claim 40, wherein the nucleic acid sequence is a double stranded sequence.

25 53. The method of claim 40, wherein said plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

54. A method of increasing the expression of a MYB transcription factor in a plant comprising:

transforming at least one plant cell with a recombinant DNA construct comprising a nucleic acid sequence encoding a MYB transcription factor; and
regenerating a transgenic plant from the transformed cell, wherein the expression of the MYB transcription factor is increased relative to a non-
5 transformed plant and whereby the increased expression of the MYB transcription factor confers enhanced stress tolerance to the plant.

55. The method of claim 54, wherein said MYB transcription factor is selected from the group consisting of MYB60, MYB74, MYB75 and MYB90.
10

56. The method of claim 54, wherein said MYB transcription factor has a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

15 57. The method of claim 54, wherein the MYB transcription factor shares a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

20 58. The method of claim 54, wherein the MYB transcription factor shares a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

25 59. The method of claim 54, wherein the MYB transcription factor shares a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

60. The method of claim 54, wherein the increased expression of the MYB transcription factor increases the stress tolerance of the plant.

61. The method of claim 60, wherein the stress tolerance comprises salt
5 stress tolerance.

62. The method of claim 60, wherein the stress tolerance comprises drought stress tolerance.

10 63. The method of claim 60, wherein the stress tolerance comprises cold stress tolerance.

64. The method of claim 60, wherein the stress tolerance comprises heat stress tolerance.
15

65. The method of claim 54, wherein the nucleic acid sequence is a DNA sequence.

66. The method of claim 54, wherein the nucleic acid sequence is an
20 RNA sequence.

67. The method of claim 54, wherein the nucleic acid sequence is a single stranded sequence.
25

68. The method of claim 54, wherein the nucleic acid sequence is a double stranded sequence.

69. The method of claim 54, wherein said plant is selected from the
30 group consisting of whole plants, plant parts and progeny thereof.

70. A method of increasing the stress tolerance of a plant comprising:
transforming at least one plant cell with a recombinant DNA construct
comprising a nucleic acid sequence encoding a MYB transcription factor; and
regenerating a transgenic plant from the transformed cell, wherein the
5 expression of the MYB transcription factor is increased relative to a non-
transformed plant and whereby the increased expression of the MYB transcription
factor confers enhanced stress tolerance to the plant.

71. The method of claim 70, wherein said MYB transcription factor is
10 selected from the group consisting of MYB60, MYB74, MYB75 and MYB90.

72. The method of claim 70, wherein said MYB transcription factor has a
sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ
ID NO:6, and SEQ ID NO:8.

15

73. The method of claim 70, wherein the MYB transcription factor shares
a sequence identity of about 50% to about 100% with an amino acid sequence
selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,
and SEQ ID NO:8.

20

74. The method of claim 70, wherein the MYB transcription factor shares
a sequence identity of about 65% to about 99% with an amino acid sequence
selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,
and SEQ ID NO:8.

25

75. The method of claim 70, wherein the MYB transcription factor shares
a sequence identity of about 70% to about 99% with an amino acid sequence
selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,
and SEQ ID NO:8.

30

76. The method of claim 70, wherein the stress tolerance comprises salt stress tolerance.

5 77. The method of claim 70, wherein the stress tolerance comprises drought stress tolerance.

78. The method of claim 70, wherein the stress tolerance comprises cold stress tolerance.

10

79. The method of claim 70, wherein the stress tolerance comprises heat stress tolerance.

80. The method of claim 70, wherein the nucleic acid sequence is a DNA
15 sequence.

81. The method of claim 70, wherein the nucleic acid sequence is an RNA sequence.

20 82. The method of claim 70, wherein the nucleic acid sequence is a single stranded sequence.

83. The method of claim 70, wherein the nucleic acid sequence is a double stranded sequence.

25

84. The method of claim 70, wherein said plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

85. A method for enhancing a plant's sensitivity to stress comprising transforming said plant with a vector as claimed in claim 22.

86. The method of claim 85, wherein said plant is selected from the
5 group consisting of whole plants, plant parts and progeny thereof.

87. A method of producing a stress sensitive transgenic plant having a reduced level of MYB transcription factors comprising:

transforming a plant with a vector comprising an antisense nucleic acid
10 molecule substantially complementary to at least a portion of a DNA molecule encoding a MYB transcription factor or at least a portion of an RNA sequence encoded by the DNA molecule encoding said MYB transcription factor; and regulatory sequences operatively linked to the antisense nucleic acid molecule;
allowing the plant to grow to at least a plantlet stage;
15 assaying the transformed plant or plantlet for altered MYB activity and/or environmental stress sensitivity; and
selecting and growing a plant having altered MYB activity and/or environmental stress sensitivity compared to a non-transformed plant.

88. The method of claim 87, wherein said MYB transcription factor is
20 selected from the group consisting of MYB60, MYB74, MYB75 and MYB90.

89. The method of claim 87, wherein said MYB transcription factor has a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ
25 ID NO:6, and SEQ ID NO:8.

90. The method of claim 87, wherein said MYB transcription factor shares a sequence identity of about 50% to about 100% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,
30 and SEQ ID NO:8.

91. The method of claim 87, wherein said MYB transcription factor shares a sequence identity of about 65% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

5

92. The method of claim 87, wherein said MYB transcription factor shares a sequence identity of about 70% to about 99% with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8.

10

93. The method of claim 87, wherein said plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

15

94. The method of claim 87, wherein said plant acts as an environmental monitor.

95. A transgenic plant produced by the transformation of at least one cell of a plant with the recombinant vector of claim 11.

20

96. The transgenic plant of claim 95, wherein the plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

97. A transgenic plant produced by the transformation of at least one cell of a plant with the recombinant vector of claim 22.

25

98. The transgenic plant of claim 97, wherein the plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

99. A seed produced by the transformation of at least one cell of a seed, plant, plant part or progeny thereof with the recombinant vector of claim 11.

100. A seed produced by the transformation of at least one cell of a seed,
5 plant, plant part or progeny thereof with the recombinant vector of claim 22.

101. A method of screening a plant for stress tolerance comprising screening the expression level of a stress tolerance-related MYB transcription factor in a plant.

10

102. The method of claim 101, wherein the plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

103. A transgenic plant stably transformed with a nucleic acid molecule
15 comprising a MYB gene, which is expressed so as to enhance stress tolerance of said plant.

104. The transgenic plant of claim 103, wherein the plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

20

105. The transgenic plant of claim 103, wherein said nucleic acid molecule further comprises a screenable marker gene.

106. A transgenic plant stably transformed with a nucleic acid molecule
25 that encodes an RNA molecule which is substantially homologous to at least a portion of an RNA transcript of a plant MYB gene, wherein said plant MYB gene hybridizes under low stringency conditions with a nucleic acid sequence encoding a MYB transcription factor selected from the group consisting of MYB60, MYB74, MYB75 and MYB90, and wherein said DNA molecule is expressed so as to enhance
30 stress sensitivity of said plant.

107. The transgenic plant of claim 106, wherein the plant is selected from the group consisting of whole plants, plant parts and progeny thereof.

5 108. The transgenic plant of claim 106, wherein said nucleic acid molecule further comprises a screenable marker gene.

109. An isolated nucleic acid molecule comprising a sequence that encodes a plant stress tolerance-related MYB transcription factor, wherein said
10 MYB transcription factor comprises a MYB transcription factor selected from the group consisting of MYB60, MYB74, MYB75 and MYB90.

110. The isolated nucleic acid molecule of claim 109, wherein the DNA molecule hybridizes under low stringency conditions with a nucleic acid sequence
15 selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7 or a variant of the isolated nucleic acid molecule.

111. The isolated nucleic acid molecule of claim 109, wherein the DNA molecule hybridizes under low stringency conditions with a nucleic acid sequence
20 encoding an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8 or a variant of the isolated nucleic acid molecule.

112. An isolated nucleic acid molecule which encodes an RNA molecule
25 which is substantially homologous to at least a portion of an RNA transcript of a plant MYB gene, wherein said plant MYB gene hybridizes under low stringency conditions with a nucleic acid sequence encoding plant stress sensitivity-related MYB transcription factor selected from the group consisting of MYB60, MYB74, MYB75 and MYB90.

30

113. The isolated nucleic acid molecule of claim 112, wherein the said plant MYB gene hybridizes under low stringency conditions with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7 or a variant of the isolated nucleic acid molecule.

5

114. The isolated nucleic acid molecule of claim 112, wherein the said plant MYB gene hybridizes under low stringency conditions with a nucleic acid sequence encoding a plant stress sensitivity-related MYB transcription factor having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8 or a variant of the isolated nucleic acid molecule.

115. A method for increasing the stress resistance of a crop in a field comprising planting in the field seeds or plants comprising transgenic plants or seeds transformed with the vector of claim 11.

15

116. A method of inhibiting the expression of MYB genes in a plant cell, said method comprising:

(1) integrating into the genome of a plant a vector comprising (a) an antisense nucleic acid molecule substantially complementary to (i) at least a portion of a DNA molecule encoding a MYB transcription factor selected from the group consisting of MYB60, MYB74, MYB75 and MYB90, or (ii) at least a portion of an RNA sequence encoded by the DNA molecule encoding said MYB transcription factor; and (b) regulatory sequences operatively linked to the antisense nucleic acid molecule such that the nucleic acid molecule is expressed in a plant cell into which it is transformed; and

20
25

(2) growing said plant, whereby said antisense nucleic acid molecule is transcribed, whereby expression of said MYB gene is inhibited.

117. A method of assaying environmental conditions of a field comprising planting at least one plant as in claim 103 in a field; and monitoring for growth of said plant.

5 118. A method of assaying environmental conditions of a field comprising planting at least one plant as in claim 106 in a field; and assaying for growth of said plant.

119. A method of increasing the production of products of the
10 phenylpropanoid biosynthesis pathway in a plant comprising:
transforming a plant cell with a recombinant DNA construct comprising a nucleic acid sequence encoding a MYB transcription factor; and
regenerating a transgenic plant from the transformed cell, wherein the expression of the MYB transcription factor increases the expression of genes
15 encoding gene products affecting the phenylpropanoid pathway, thereby increasing the production of products of the phenylpropanoid biosynthesis pathway.

120. The method of claim 119, wherein the products of the phenylpropanoid pathway include one or more of stilbenes, flavonoids, lignins,
20 salicylic acid, anthocyanins, and phenolic derivatives.

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AtMYB60

- cDNA = 949 bp
- Peptide = 281 aa

1 GAGAGAGAAAGATGGGTAGGCCTCCATGCTGTGACAAGATAGGGATCAAGAAAGGACCAT
M G R P P C C D K I G I K K G P W

61 GGA CTCTGAAGAAGATATCATTCTTGTCTTACATTCAAGAACATGGTCCTGGAAACT
T P E E D I I L V S Y I Q E H G P G N W

121 GGAGATCAGTTCCCAACACTGGGTTATTGAGATGCAGCAAAAGTTGTAGACTGAGAT
R S V P T N T G L L R C S K S C R L R W

181 GGACAAATTATCTGAGACCTGGAATTAACGTGGAACTTTACTCCTCATGAAGAAGGAA
T N Y L R P G I K R G N F T P H E E G M

241 TGATCATTCAC TTGCAAGCCTTATTGGGTAACAAATGGGCGTCCATAGCTTCATACCTAC
I I H L Q A L L G N K W A S I A S Y L P

301 CACAAAGAACGGACAATGATATCAAGAACTACTGGAACACACATTTAAAGAAGAAGCTCA
Q R T D N D I K N Y W N T H L K K K L N
MYB60FII

361 ACAAGTCTGACAGTGATGAGAGGAGCAGATCAGAGAACATTGCGCTGCAAACTTCTTCGA
K S D S D E R S R S E N I A L Q T S S T

421 CAAGAAACACCATTAATCATAGATCTACCTATGCTTCAAGCACAGAAAACATTTCCCGCC
R N T I N H R S T Y A S S T E N I S R L

481 TTGTGGAGGGTTGGATGAGAGCGTCTCCAAAGAGTAGTACAAGTACTACTTTCTTGGAAC
V E G W M R A S P K S S T S T T F L E H

541 ACAAATGCAGAACCGGACAAACAATTTATCGATCATCACAGTGATCAGTTTCCATACG
K M Q N R T N N F I D H H S D Q F P Y E

601 AGCAGCTTCAAGGTTCTAGGGAAGAGGGTCATAGCAAAGGAATCAACGGGGATGATGACC
Q L Q G S R E E G H S K G I N G D D D Q

661 AGGGTATAAAGAATTGAGAGAATAACAACGGTGATGATGTTTCATCATGAAGATGGTGATC
G I K N S E N N N G D D V H H E D G D H

721 ATGAGGATGATGATGATCATAATGCAACACCACCATTGACATTTATTGAGAAATGGCTTT
E D D D D H N A T P P L T F I E K W L L
MYB60RIII

781 TGGAGGAAACAAGTACTACTGGGGGTCAAATGGAAGAGATGAGCCACTTGATGGAGCTCT
E E T S T T G G Q M E E M S H L M E L S

841 CTAATATGCTTTAATTGTGACATTTCTCCTTTATTTTTCTTTATCCTTGTGAATCTTA
N M L *

901 TAAATGAGACTACTAATTTTATATACACAAATAAAGAAACCAGAAAGAC

FIG. 1

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AtMYB74

- cDNA = 901 bp
- Peptide = 260 aa

1 TATTAAGCGTGAAGATTCTCTTTTGAAGAAGAAGAAACCATTATTCAACTTCACGGCAT
I K R G R F S F E E E E T I I Q L H G I

61 CATGGGAAACAAGTGGTCTGCGATTGCGGCTCGTTTGCCTGGAAGAACAGACAACGAGAT
M G N K W S A I A A R L P G R T D N E I
MYB74FII

121 CAAAACTATTGGAACACTCACATCAGAAAAAGACTTCTAAAGATGGGAATCGACCCGGT
K N Y W N T H I R K R L L K M G I D P V

181 TACACAACTCCACGTCTTGATCTTCTCGATATCTCCTCCATTCTCAGCTCATCTATCTA
T H T P R L D L L D I S S I L S S S I Y

241 CAACTCTTCGCATCATCATCATCATCAACAACATATGAACATGTCGAGGCTCAT
N S S H H H H H H H Q Q H M N M S R L M

301 GATGAGTGATGGTAATCATCAACCATTGGTTAACCCCGAGATACTCAAACCTCGCAACCTC
M S D G N H Q P L V N P E I L K L A T S

361 TCTCTTTTCAAACCAAACCAACCAACACACGAGAACAACACGGTTAACCAAAC
L F S N Q N H P N N T H E N N T V N Q T

421 CGAAGTAAACCAATACCAAACCGGTTACAACATGCCTGGTAATGAAGAATTACAATCTTG
E V N Q Y Q T G Y N M P G N E E L Q S W

481 GTTCCCTATCATGGATCAATTCACGAATTTCCAAGACCTCATGCCAATGAAGACGACGGT
F P I M D Q F T N F Q D L M P M K T T V

541 CCAAATTCATTGTCATACGATGATGATTGTTGGAAGTCCAATTTTGTATTAGAACCTTA
Q N S L S Y D D D C S K S N F V L E P Y

601 TTACTCCGACTTTGCTTCAGTCTTGACCACACCTTCTTCAAGCCCGACTCCGTAAACTC
Y S D F A S V L T T P S S S P T P L N S
MYB74RIII

661 AAGTTCCTCAACTTACATCAATAGTAGCACTTGCAGCACCGAGGATGAAAAAGAGAGTTA
S S S T Y I N S S T C S T E D E K E S Y

721 TTACAGTGATAATATCACTAATTATTCGTTTGATGTTAATGGTTTTCTCCAATTCCAATA
Y S D N I T N Y S F D V N G F L Q F Q *

781 AACAAAACGCCATTGGAATAGAGTTATGTAAACATGCAATCATTGTATTTGTTATATAGA
841 TTTTGTTACATATCCAAAATCCAAAATACTATAGTTTTAAAAATAAAAAAAAAAAAAAAAAA
901 A

FIG.2

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AtMYB 75 cDNA sequence

CCACGCGTCCGTACCTTTTACAATTTGTTTATATATTTTACGTATCTATCTTTGTTCCATG
GAGGGTTCGTCCAAAGGGCTGCGAAAAGGTGCTTGGACTACTGAAGAAGATAGTCTCTTGA
GACAGTGCATTAATAAGTATGGAGAAGGCCAATGGCACCAAGTTCCTGTAAGAGCTGGGCT
AAACCGGTGCAGGAAAAGTTGTAGATTAAGATGGTTGAAGTATTTGAAGCCAAGTATCAAG
AGAGGAAAACCTTAGCTCTGATGAAGTCGATCTTCTTCTTCGCCTTCATAGGCTTCTAGGGA
ATAGGTGGTCTTTAATTGCTGGAAGATTACCTGGTCGGACCGCAAATGACGTCAAGAATTA
CTGGAACACTCATCTGAGTAAGAAACATGAACCGTGTTGTAAGATAAAGATGAAAAAGAGA
GACATTACGCCCATTCTACAACACCGGCACTAAAAACAATGTTTATAAGCCTCGACCTC
GATCCTTCACAGTTAAACAAGACTGCAACCATCTCAATGCCCCACCAAAGTTGACGTTAA
TCCTCCATGCCTTGGACTTAACATCAATAATGTTTGTGACAATAGTATCATATACAACAAA
GATAAGAAGAAAGACCAACTAGTGAATAATTTGATTGATGGAGATAATATGTGGTTAGAGA
AATTCCTAAGGAAAGCCAAGAGGTAGATATTTTGGTTCCTGAAGCGACGACAACAGAAAAG
GGGGACACCTTGGCTTTTGACGTTGATCAACTTTGGAGTCTTTTCGATGGAGAGACTGTGA
AATTTGATTAGTGTTCGAACATTTGTTTGCCTTGTGTATAGGTTTGCTTTCACCTTTTA
ATTTGTGTGTTTTGATAAATAAGCTAATAGTTTTTAGCATTTTAATGAAATATTTCAAGTT
TCCGTGTTAC

FIG.3A

Aminoacid sequence of AtMYB 75

MEGSSKGLRKGAWTTEEDSLLRQCINKYGEGKWHQVPVRAGLNRCRKSCRLRWLNLYLKPSIK
RGKLSSDEVLLLLRLHRLLGNRWSLIAGRLPGRTANDVKNYWNTHLSKKHEPCCKIKMKKRD
ITPIPTTPALKNNVYKPRPRSFTVNNDCNHLNAPPKVDVNPPCLGLNINNVCDSHYNKDQK
KDQLVNNLIDGDNMWLEKFLRKAKR

FIG.3B

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AtMYB90

- cDNA = 1043 bp
- Peptide = 250 aa

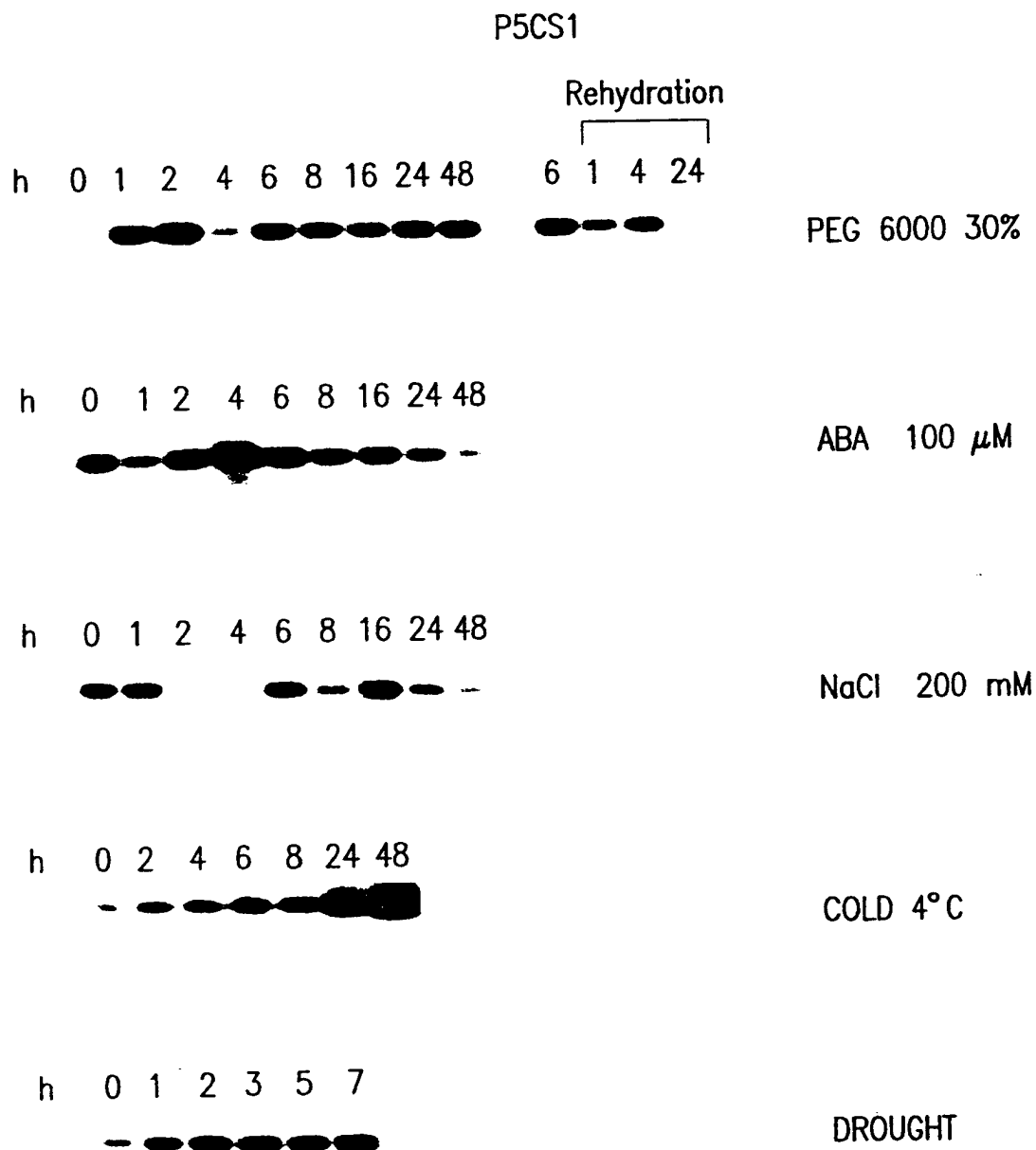
```

1  GTCGACCCACGCGTCCGTGGGAAGCCACAATAACCCCCTATTCCTCGGCCTTTTTTAAAA
61  AAGTTTTAGAATAATCCGATAAAATACTTTTATATTAATTTTTCTTTGGTCCATGGAGGG
    M E G
121 TTCGTCAAAGGGTTGAGGAAAGGTGCATGGACTGCTGAAGAAGATAGTCTCTTGAGGCT
    S S K G L R K G A W T A E E D S L L R L
181 ATGTATTGATAAGTATGGAGAAGGCAAATGGCATCAAGTTCCTTTGAGAGCTGGGCTAAA
    C I D K Y G E G K W H Q V P L R A G L N
241 TCGATGCAGAAAGAGTTGTAGACTAAGATGGTTGAACTATTTGAAGCCAAGTATCAAGAG
    R C R K S C R L R W L N Y L K P S I K R
301 AGGAAGACTTAGCAATGATGAAGTTGATCTTCTTTCGCCTTCATAAGCTTCTAGGAAA
    G R L S N D E V D L L L R L H K L L G N
361 TAGGTGGTCCTTGATTGCTGGTCGATTGCCTGGTCGGACCGCTAATGATGTCAAAAATTA
    R W S L I A G R L P G R T A N D V K N Y
    EST193FB
421 CTGGAACACCCATCTGAGTAAAAAACATGAGTCTTCGTGTTGTAAGTCTAAAATGAAAAA
    W N T H L S K K H E S S C C K S K M K K
481 GAAAAACATTATTTCCCTCCTACAACACCGGTCCAAAAAATCGGTGTTTTTAAGCCTCG
    K N I I S P P T T P V Q K I G V F K P R
541 ACCTCGATCCTTCTCTGTTAACAATGGTTGCAGCCATCTCAATGGTCTGCCAGAAGTTGA
    P R S F S V N N G C S H L N G L P E V D
601 TTTAATTCCTTCATGCCTTGGACTCAAGAAAAATAATGTTTGTGAAAATAGTATCACATG
    L I P S C L G L K K N N V C E N S I T C
661 TAACAAAGATGATGAGAAAGATGATTTTGTGAATAATCTAATGAATGGAGATAATATGTG
    N K D D E K D D F V N N L M N G D N M W
721 GTTGGAGAATTTACTGGGGGAAAACCAAGAAGCTGATGCGATTGTTCTGAAGCGACGAC
    L E N L L G E N Q E A D A I V P E A T T
    MYB90RIII
781 AGCTGAACATGGGGCCACTTTGGCGTTTGACGTTGAGCAACTTTGGAGTCTGTTTCATGG
    A E H G A T L A F D V E Q L W S L F D G
841 AGAGACTGTTGAACCTGATTAGTGTTTCTACCGTTTGTTTAAGATTGTGGGTGGCTTTT
    E T V E L D *
901 CTTTCGTATTTTAGTAATGTATTTTTCTGTATGAAGTAAAGAATTTAGCATTTTAAGAA
961 AAATGGTTATGTTTCTACGTAATAAAAAAACGTTATTTATAAAAAAAAAAAAAAAAAAAAA
1021 AAAAAAAAAAAAAAGGGCGGCCGC

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FIG.4

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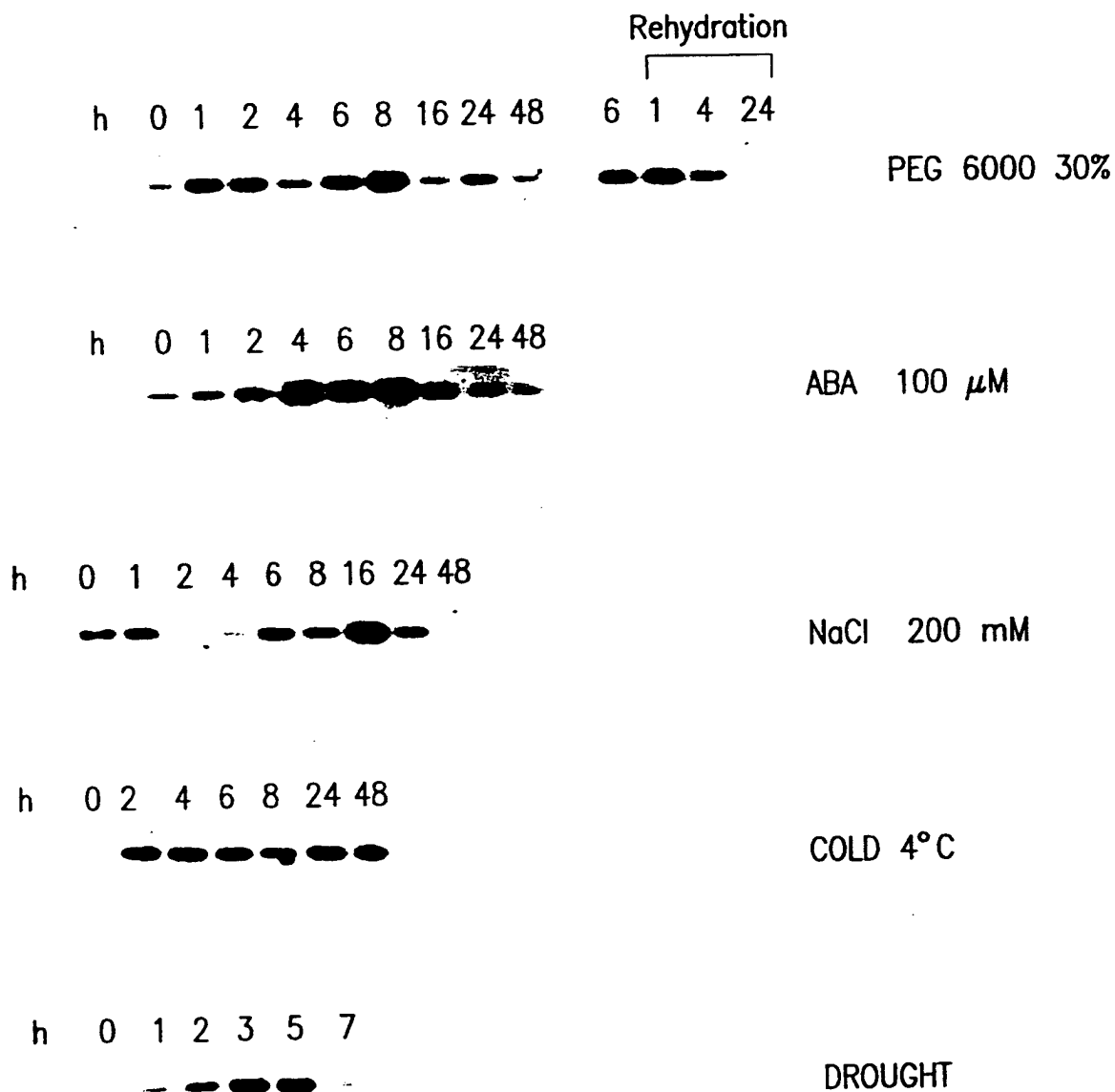
RT-PCR analysis

FIG.5

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RD22



RT-PCR analysis

FIG.6

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ERD10

Reidratazione

h 0 1 2 4 6 8 16 24 48

6 1 4 24



PEG 6000 30%

h 0 1 2 4 6 8 16 24 48

ABA 100 μ M

h 0 1 2 4 6 8 16 24 48



NaCl 200 mM

h 0 2 4 6 8 24 48



COLD 4°C

h 0 1 2 3 5 7



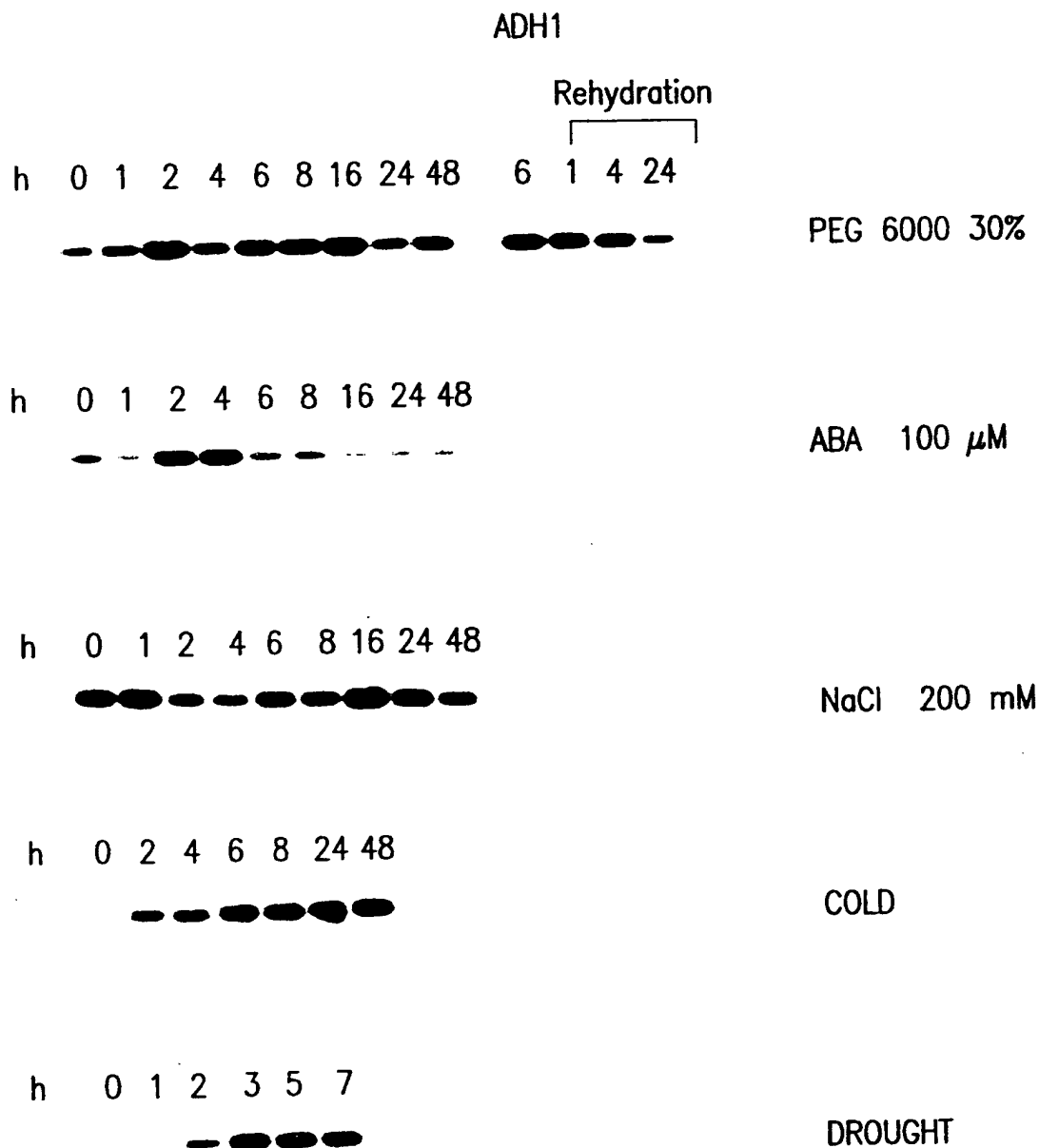
DROUGHT

RT-PCR analysis

FIG.7

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RT-PCR analysis

FIG.8

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AtMYB74

Rehydration

h 0 1 2 4 6 8 16 24 48



6 1 4 24



PEG 6000 30%

h 0 1 2 4 6 8 16 24 48



ABA 100 μM

h 0 1 2 4 6 8 16 24 48



NaCl 200 mM

h 0 2 4 6 8 24 48



COLD 4°C

h 0 1 2 3 5 7



DROUGHT

RT-PCR analysis

FIG.9

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AtMYB75

Rehydration

h 0 1 2 4 6 8 16 24 48

6 1 4 24



PEG 6000 30%

h 0 1 2 4 6 8 16 24 48



ABA 100 μ M

h 0 1 2 4 6 8 16 24 48



NaCl 200 mM

h 0 2 4 6 8 24 48

...

COLD 4°C

h 0 1 2 3 5 7



DROUGHT

RT-PCR analysis

FIG.10

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COLD 4°C
h 0 2 4 6 8 24 48

PEG 6000 30%

Reidratazione

6 1 4 24

h 0 1 2 4 6 8 16 24 48

AtMYB75

h

Reidratazione

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h 0 1 2 4 6 8 16 24 48

AtMYB74

h 0 2 4 6 8 24 48

ERD10

ADH1

P5CS1

RD22

FIG. 11

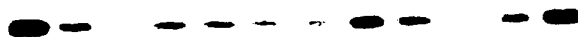
12/16

AtMYB90

Reidratazione

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6 1 4 24



PEG 6000 30%

h 0 1 2 4 6 8 16 24 48



ABA 100 μ M

h 0 1 2 4 6 8 16 24 48



NaCl 200 mM

h 0 2 4 6 8 24 48



COLD 4°C

h 0 1 2 3 5 7



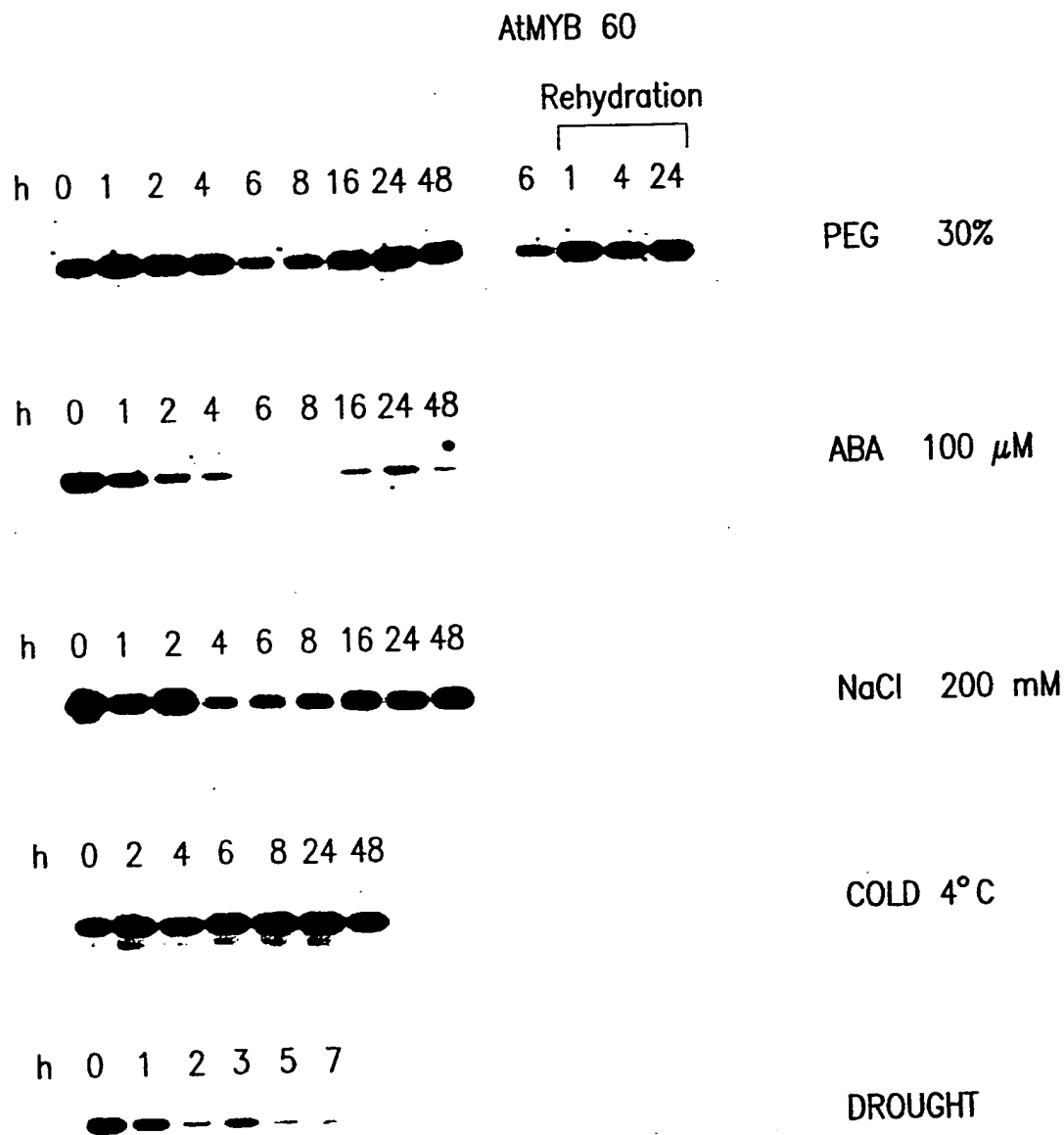
DROUGHT

RT-PCR analysis

FIG.12

SUBSTITUTE SHEET (RULE 26)

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RT-PCR analysis

FIG.13

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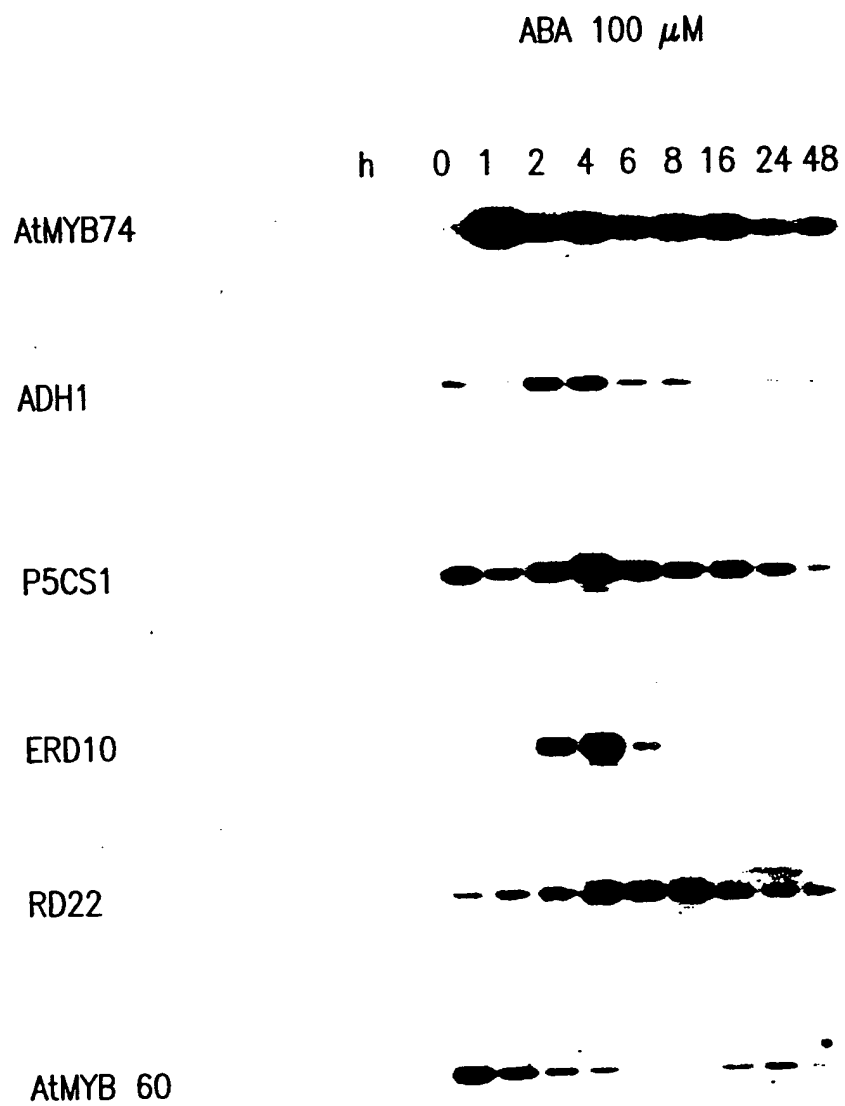


FIG. 14

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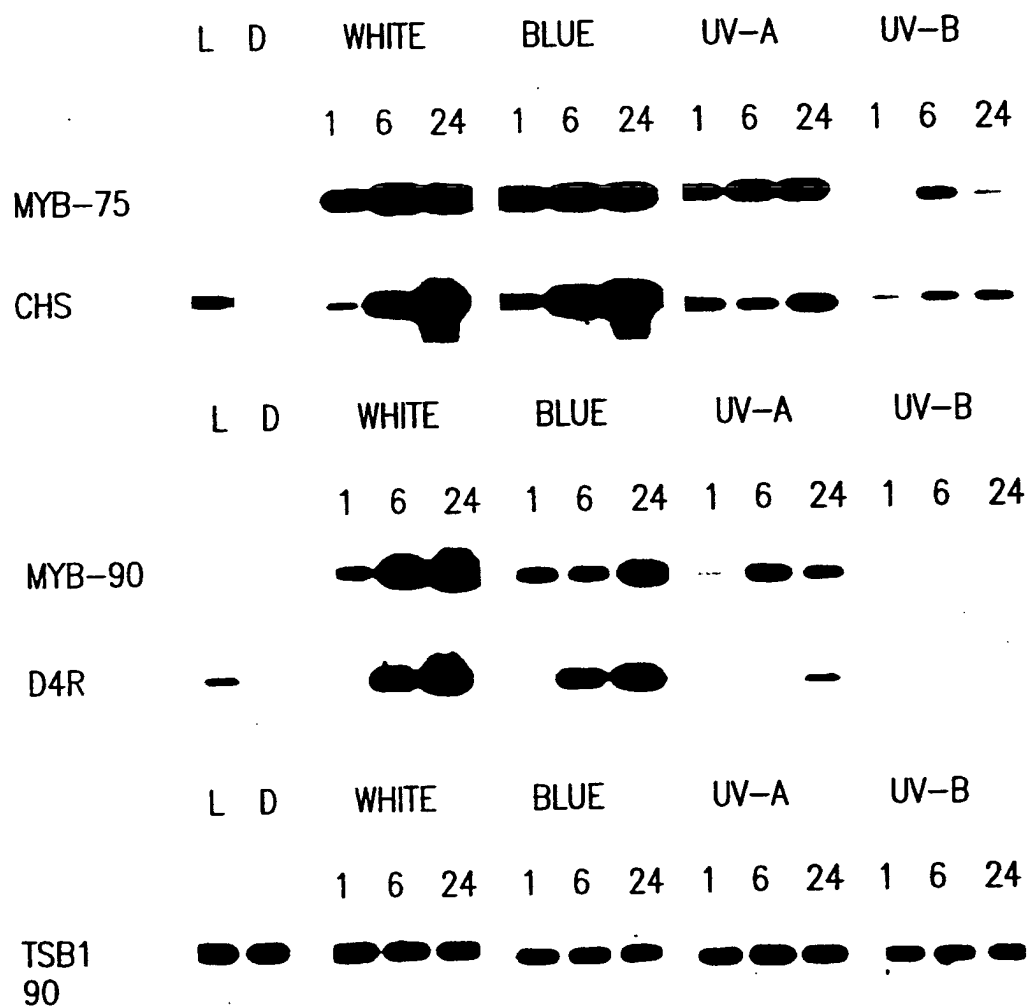


FIG.15

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PHENYLPROPANOID METABOLIC PATHWAY

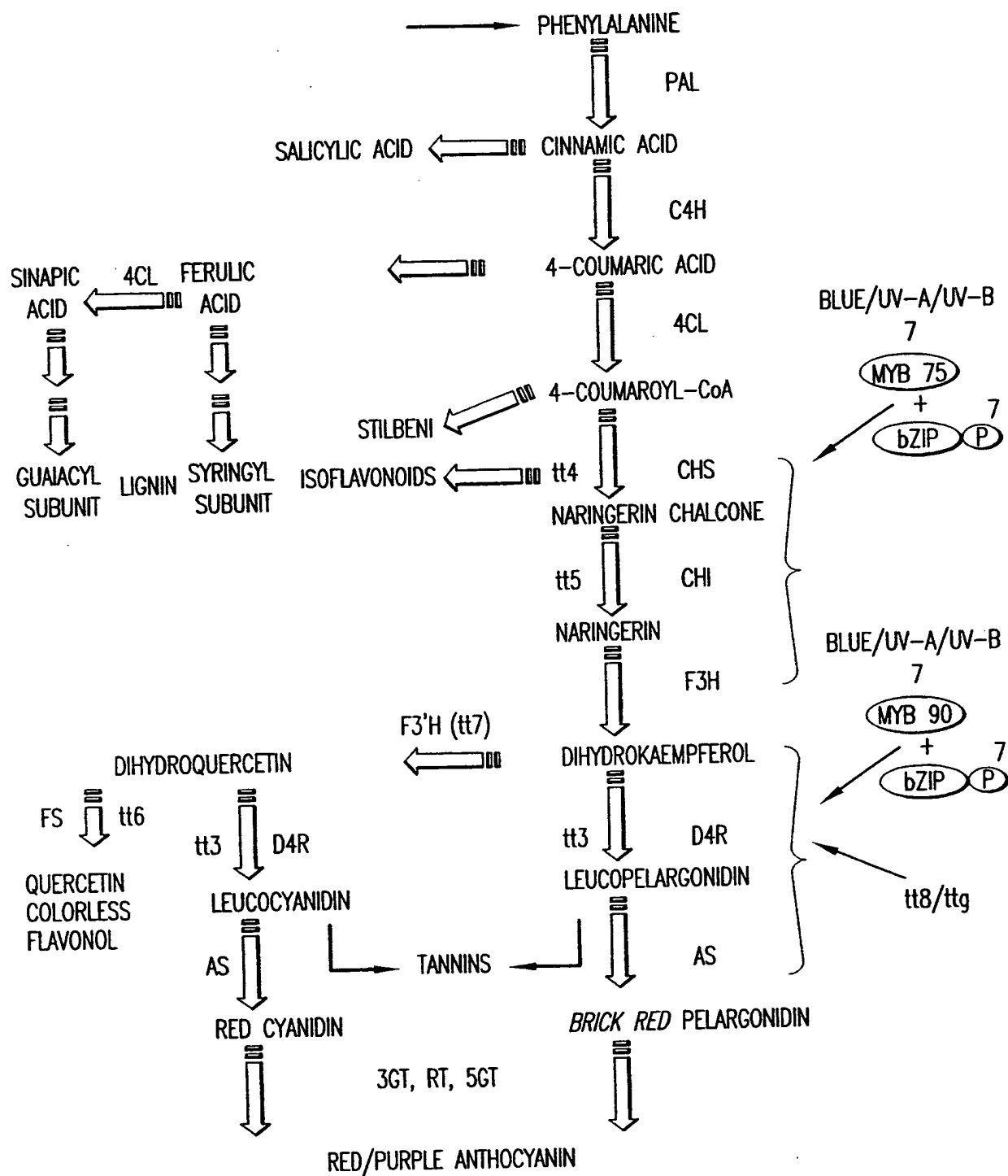


FIG. 16

SUBSTITUTE SHEET (RULE 26)

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Thereof

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      15              20              25

caa gaa cat ggt cct gga aac tgg aga tca gtt ccc acc aac act ggg 146
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      30              35              40              45

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tca tac cta cca caa aga acg gac aat gat atc aag aac tac tgg aac 338
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cac agt gat cag ttt cca tac gag cag ctt caa ggt tct agg gaa gag	626			
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Cys Ser Lys Ser Cys Arg Leu Arg Trp Thr Asn Tyr Leu Arg Pro Gly	
50 55 60	
Ile Lys Arg Gly Asn Phe Thr Pro His Glu Glu Gly Met Ile Ile His	
65 70 75 80	
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 Asn Ile Ala Leu Gln Thr Ser Ser Thr Arg Asn Thr Ile Asn His Arg
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 Ser Thr Tyr Ala Ser Ser Thr Glu Asn Ile Ser Arg Leu Val Glu Gly
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 165 170 175
 His Lys Met Gln Asn Arg Thr Asn Asn Phe Ile Asp His His Ser Asp
 180 185 190
 Gln Phe Pro Tyr Glu Gln Leu Gln Gly Ser Arg Glu Glu Gly His Ser
 195 200 205
 Lys Gly Ile Asn Gly Asp Asp Gln Gly Ile Lys Asn Ser Glu Asn
 210 215 220
 Asn Asn Gly Asp Asp Val His His Glu Asp Gly Asp His Glu Asp Asp
 225 230 235 240
 Asp Asp His Asn Ala Thr Pro Pro Leu Thr Phe Ile Glu Lys Trp Leu
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 cct gga aga aca gac aac gag atc aaa aac tat tgg aac act cac atc 145
 Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Ile
 35 40 45
 aga aaa aga ctt cta aag atg gga atc gac ccg gtt aca cac act cca 193
 Arg Lys Arg Leu Leu Lys Met Gly Ile Asp Pro Val Thr His Thr Pro
 50 55 60
 cgt ctt gat ctt ctc gat atc tcc tcc att ctc agc tca tct atc tac 241
 Arg Leu Asp Leu Leu Asp Ile Ser Ser Ile Leu Ser Ser Ser Ile Tyr
 65 70 75 80
 aac tct tcg cat cat cat cat cat cat cat caa caa cat atg aac atg 289
 Asn Ser Ser His His His His His His His Gln Gln His Met Asn Met
 85 90 95
 tcg agg ctc atg atg agt gat ggt aat cat caa cca ttg gtt aac ccc 337
 Ser Arg Leu Met Met Ser Asp Gly Asn His Gln Pro Leu Val Asn Pro
 100 105 110

gag ata ctc aaa ctc aac ctc tct ctc ttt tca aac caa aac cac ccc 385
 Glu Ile Leu Lys Leu Asn Leu Ser Leu Phe Ser Asn Gln Asn His Pro
 115 120 125

aac aac aca cac gag aac aac acg gtt aac caa acc gaa gta aac caa 433
 Asn Asn Thr His Glu Asn Asn Thr Val Asn Gln Thr Glu Val Asn Gln
 130 135 140

tac caa acc ggt tac aac atg cct ggt aat gaa gaa tta caa tct tgg 481
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ttc cct atc atg gat caa ttc acg aat ttc caa gac ctc atg cca atg 529
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aag acg acg gtc caa aat tca ttg tca tac gat gat gat tgt tgc aag 577
 Lys Thr Thr Val Gln Asn Ser Leu Ser Tyr Asp Asp Asp Cys Ser Lys
 180 185 190

tcc aat ttt gta tta gaa cct tat tac tcc gac ttt gct tca gtc ttg 625
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acc aca cct tct tca agc ccg act ccg tta aac tca agt tcc tca act 673
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 Tyr Ser Asp Asn Ile Thr Asn Tyr Ser Phe Asp Val Asn Gly Phe Leu
 245 250 255

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 Arg Leu Asp Leu Leu Asp Ile Ser Ser Ile Leu Ser Ser Ser Ile Tyr
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 Asn Ser Ser His His His His His His Gln Gln His Met Asn Met
 85 90 95
 Ser Arg Leu Met Met Ser Asp Gly Asn His Gln Pro Leu Val Asn Pro

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 gatgaaaaag agagacatta cgccatttcc tacaacaccg gcaactaaaa acaatgttta 480
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 Cys Arg Leu Arg Trp Leu Asn Tyr Leu Lys Pro Ser Ile Lys Arg Gly
 50 55 60
 Lys Leu Ser Ser Asp Glu Val Asp Leu Leu Leu Arg Leu His Arg Leu
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 Leu Gly Asn Arg Trp Ser Leu Ile Ala Gly Arg Leu Pro Gly Arg Thr

85 90 95
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 100 105 110
 Glu Pro Cys Cys Lys Ile Lys Met Lys Lys Arg Asp Ile Thr Pro Ile
 115 120 125
 Pro Thr Thr Pro Ala Leu Lys Asn Asn Val Tyr Lys Pro Arg Pro Arg
 130 135 140
 Ser Phe Thr Val Asn Asn Asp Cys Asn His Leu Asn Ala Pro Pro Lys
 145 150 155 160
 Val Asp Val Asn Pro Cys Leu Gly Leu Asn Ile Asn Asn Val Cys
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Ala Asn Asp Val Lys Asn Tyr Trp Asn Thr His Leu Ser Lys Lys His
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 Val Asn Asn Leu Met Asn Gly Asp Asn Met Trp Leu Glu Asn Leu Leu
 195 200 205
 Gly Glu Asn Gln Glu Ala Asp Ala Ile Val Pro Glu Ala Thr Thr Ala
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16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/30503

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) A01H 1/00, 9/00, 11/00; C07H 21/04; C12N 5/04, 5/10, 15/00, 15/09, 15/63, 15/70, 15/74, 15/82, 15/87
US CL 435/ 320.1, 419, 468; 536/ 23.6; 800/ 278, 295

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/ 320.1, 419, 468; 536/ 23.6; 800/ 278, 295

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	KRANZ et al. Towards functional characterisation of the members of the R2R3-MYB gene family from Arabidopsis thaliana. The Plant Journal. 1998, Vol. 16, No. 2, pages 263-276, especially pages 264-267 Table 1, page 271 column 2 2nd full paragraph, pages 272-273 Table 2.	1-22, 109-114 38-86, 95-100, 103-108, 115, 119-120
X --- Y	GenBank, Accession AF062895, KRANZ et al. Towards functional characterisation of the members of the R2R3-MYB gene family from Arabidopsis thaliana. The Plant Journal. 1998, Vol. 16, No. 2, pages 263-276, especially page 266 Table 1 (X and Y).	1-22, 109-114 38-86, 95-100, 103-108, 115, 119-120
X --- Y	GenBank, Accession AF062907, KRANZ et al. Towards functional characterisation of the members of the R2R3-MYB gene family from Arabidopsis thaliana. The Plant Journal. 1998, Vol. 16, No. 2, pages 263-276, especially page 266 Table 1 (X and Y).	1-22, 109-114 38-86, 95-100, 103-108, 115, 119-120
X --- Y	GenBank, Accession AF062908, KRANZ et al. Towards functional characterisation of the members of the R2R3-MYB gene family from Arabidopsis thaliana. The Plant Journal. 1998, Vol. 16, No. 2, pages 263-276, especially page 266 Table 1 (X and Y).	1-22, 109-114 38-86, 95-100, 103-108, 115, 119-120



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

16 January 2001 (16.01.2001)

Date of mailing of the international search report

07 MAR 2001

Name and mailing address of the ISA/US

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Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/30503

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	GenBank, Accession AF062915, KRANZ et al. Towards functional characterisation of the members of the R2R3-MYB gene family from Arabidopsis thaliana. The Plant Journal. 1998, Vol. 16, No. 2, pages 263-276, especially page 267 Table 1 (X and Y).	1-22, 109-114 ----- 38-86, 95-100, 103-108, 115, 119-120
P,Y	SHIMIZU et al. Molecular cloning and characterization of a subfamily of UV-B responsive MYB genes from soybean. Breeding Science. June 2000, Vol. 50, pages 81-90, especially page 83 Figure 1, page 86 column 2 first and second full paragraphs, page 87 Figure 3, page 88 Figure 5, page 89 Figure 6.	1-22, 38-86, 95-100, 103-115, 119-120
Y	SHINOZAKI et al. Molecular responses to water stress in Arabidopsis thaliana. J. Plant Res. June 1998, Vol. 111, pages 345-351, especially page 347 column 2 first full paragraph, page 348 Figure 4.	38-86, 95-100, 103-108, 115, 119-120
X --- Y	KIRIK et al. Ectopic expression of a novel MYB gene modifies the architecture of the Arabidopsis inflorescence. The Plant Journal. March 1998, Vol. 13, No. 6, pages 729-742, especially page 731 Figure 1, page 734 Figure 5, page 736 Figure 7, page 737 paragraph spanning columns 1 and 2, page 740 Vector constructs and plant transformation.	95-100, 103-108 ----- 1-22, 38-86, 109-115, 119-120
Y	ABE et al. Role of Arabidopsis MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. The Plant Cell. October 1997, Vol. 9, pages 1859-1868, especially page 1863 Figure 4A, page 1865 Figure 6 and column 2 second full paragraph, page 1866 Figure 7.	38-86, 95-100, 103-115, 119-120
Y	LOIDL et al. Oncogene- and tumor-suppressor gene-related proteins in plants and fungi. Critical Reviews in Oncogenesis. 1996, Vol. 7, Nos: 1 and 2, pages 49-64, especially pages 51-52 A. Myb-Related Genes/Proteins, page 52 Table 1.	38-86, 95-100, 103-108, 115, 119-120
Y	ITURRIAGA et al. A family of novel myb-related genes from the resurrection plant Craterostigma plantagineum are specifically expressed in callus and roots in response to ABA or dessication. Plant Molecular Biology. November 1996, Vol. 32, pages 707-716, especially pages 711 Figure 3, page 713 Figure 6, page 114.	1-22, 38-86, 95-100, 103-115, 119-120
Y	SCHAEFFER et al. Identification of enhancer and silencer regions involved in salt-responsive expression of Crassulacean acid metabolite (CAM) genes in the facultative halophyte Mesembryanthemum crystallinum. Plant Molecular Biology. May 1995, Vol. 28, pages 205-218, especially page 209 Figure 1, page 213 Figure 4, page 215 column 2 first full paragraph, page 216 column 1 paragraph spanning pages 215-216 and paragraph spanning columns 1 and 2.	38-86, 95-100, 103-108, 115, 119-120
Y	YAMAGUCHI-SHINOZAKI et al. Regulation of genes that are induced by drought stress in Arabidopsis thaliana. J. Plant Research. 1995, Vol. 108, pages 127-136, especially page 128 Figure 1, page 133 column 2 first paragraph - page 135 column 1 first paragraph.	1-22, 38-86, 95-100, 103-115, 119-120
Y	YAMAGUCHI-SHINOZAKI et al. Function and regulation of genes that are induced by dehydration stress in Arabidopsis thaliana. JIRCAS Journal. 1994, Vol. 1, pages 69-79, entire article.	1-22, 38-86, 95-100, 103-115, 119-120
Y	URAO et al. An Arabidopsis myb homolog is induced by dehydration stress and its gene product binds to the conserved MYB recognition sequence. The Plant Cell. November 1993, Vol. 5, pages 1529-1539, especially page 1530 Figure 1, page 1531 Figure 2, page 1532 Figures 3 and 4.	1-22, 38-86, 95-100, 103-115, 119-120

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INTERNATIONAL SEARCH REPORT

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-22, 38-86, 95-100, 103-115, 119-120

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.
No protest accompanied the payment of additional search fees.

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BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-22, 38-86, 95-100, 103-115, 119, and 120, drawn to compounds comprising a MYB nucleic acid molecule.

Group II, claim(s) 23-27, drawn to MYB polypeptides.

Group III, claim(s) 28-32, drawn to antibodies against MYB polypeptides.

Group IV, claim(s) 33-37, drawn to variant MYB polypeptides.

Group V, claim(s) 87-94, drawn to a method of producing a stress sensitive transgenic plant.

Group VI, claim(s) 101-102, drawn to a method of screening a plant for stress tolerance.

Group VII, claim(s) 117-118, drawn to a method of assaying environmental conditions of a field.

Group VIII, claim(s) 116, drawn to a method of inhibiting the expression of MYB genes in a plant cell.

The inventions listed as Groups I-VIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Groups I-VIII lack the same special technical feature in that the products differ structurally and functionally from one another, and the methods result in different products or uses. The products of Group I are compounds comprising a MYB nucleic acid molecule, which is not a special technical feature of the products of Groups II-IV. The products of Group II are MYB polypeptides, which is not a special technical feature of the products of Groups I, III, and IV. The products of Group III are antibodies against MYB polypeptides, which is not a special technical feature of the products of Groups I, II, and IV. The products of Group IV are variant MYB polypeptides, which is not a special technical feature of the products of Groups I-III. The method of Group V is used to make stress sensitive plants, which is not a special technical feature of the methods of Groups VI-VIII. The method of Group VI is used to identify stress tolerant plants, which is not a special technical feature of the methods of Groups V, VII, and VIII. The method of Group VII is used to identify the environmental conditions of a field, which is not a special technical feature of the methods of Groups V, VI, and VIII. The method of Group VIII is used to inhibit MYB gene expression, which is not a special technical feature of the methods of Groups V-VII. Therefore, lack of unity between the stated groups is properly made.

Continuation of B. FIELDS SEARCHED Item 3: WEST & STN(AGRICOLA, BIOSIS, BIOTECHNO, BIOTECHDS, BIOTECHABS, CABA, CAPLUS, EMBASE, MEDLINE, SCISEARCH) search terms: plant transcription factor, myb, Arabidopsis, stress, inventor name ; STIC SEQUENCE SEARCH SEQ ID NOS:1-8.

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